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STUDIES

FROM

THE SARANAC LABORATORY FOR THE STUDY OF TUBERCULOSIS

E. L. TRUDEAU, M.D., DIRECTOR

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1904–1910

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E. L. Trudeau, M.D., *Director*.

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Appendix — Description of Cultures, Etc.

(These studies were made possible by the generosity of Mrs. A. A. Anderson, of New York City.)

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STUDIES IN IMMUNITY TO TUBERCULOSIS.

I. (*a.*) HYPERSUSCEPTIBILITY OR ANAPHYLAXIS.*

EDWARD R. BALDWIN, M.D.

(*From the Saranac Laboratory for the Study of Tuberculosis; E. L. Trudeau, M.D., Director.*)

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* Received for publication Jan. 24, 1910.

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INTRODUCTION. — From the time of the discovery by Koch of the extraordinary sensitiveness of tuberculous animals to tuberculin, many contributions have been made to the literature of the subject, and many experiments performed for the purpose of explaining the phenomenon.

Analogous reactions were recognized, as in the case of mallein for glanders, and yet the true significance of these was not understood until the clinical study of Pirquet and Schick on "Serum Disease" (1903). These authors called attention to the general law which governed the response of the animal tissues to the introduction of foreign protein substances. They saw the correspondence between the prompt local reaction to repeated serum injections and that to tuberculin. Under the general term "Allergie" or "changed reaction" von Pirquet classed the phenomena following re-vaccination in vaccinia, and reinoculation with tubercle bacilli in a previously infected individual. The immediate inflammatory reaction betokened a change in the tissues acquired from a previous exposure to the same substances. The nature of this change was explained by Wolff-Eisner (1904) as similar to that produced in an animal immunized against typhoid bacilli, *i.e.*, the development of lysins which are capable of setting free toxic, irritant products of digestion — endotoxins. These in turn accounted for the reaction. A different explanation was given by Bail (1904) for the fatal toxic effects of reinoculations of tubercle bacilli in previously tuberculous animals. He assumed an "aggressin" or special

secretion from the bacilli called forth by contact with the animal tissues. The experiments of J. Woods Price,[1] in association with the writer, failed to support this theory in so far as it related to acute poisoning. Fatal poisoning of tuberculous animals is well known to follow doses of tuberculin or dead bacilli in comparatively small doses, while healthy animals withstand enormous amounts except when very young.

When the anaphylactic death of serum-sensitized animals was first noted in 1904 by Theobald Smith,[2] and the prolific study of these phenomena was entered upon, experiments were naturally undertaken with tubercle bacilli. The first to do this were Rosenau and Anderson,[2] who published in 1907 some attempts to sensitize guinea-pigs with saline extracts of tubercle bacilli. After thirty-two days they tested their animals with large doses (six cubic centimeters and ten cubic centimeters) of extract intraperitoneally without obtaining fatal or marked symptoms of anaphylaxis except in one instance. They also mention the probability of maternal transmission of this susceptibility, which they demonstrated to be possible in serum sensitization.

The symptoms of anaphylaxis from various bacterial proteins appeared milder as a rule in their experiments than from serum proteins, and required larger quantities to confer sensitivity, allowances being made for differences in concentration of the protein. The analogy was, however, sufficiently demonstrated by Rosenau and Anderson, and it appeared of interest to study the especial phases which present themselves in connection with tuberculosis.

Very interesting contributions closely related to this work have been made by V. C. Vaughan[3] and his associates with products of tubercle bacilli and other bacteria, split off by alcohol-alkali treatment. In this way a non-toxic but sensitizing substance and a toxic but non-sensitizing substance are obtained. The poisonous symptoms appear not to differ from those seen in acute serum anaphylaxis in which the respiratory mechanism is gravely affected, and are evidently related in some way. These studies are especially

important in offering an explanation of the mechanism of protein poisoning.

During the past three years the general subject of protein sensitization has been the object of diligent research, but it would not be useful in this place to review the literature, since it may be found elsewhere in very complete form.[4, 5, 6] The writer has already reviewed tuberculous hypersensitization in part (Yale Medical Alumni Lectures, 1909[7]).

Previously it had been noted in our experiments that large animals (horses, asses, and calves), undergoing immunization for the production of antitoxic sera for tuberculosis, occasionally died suddenly during or soon after the injections given intravenously. This was also noted rarely in rabbits which were being injected repeatedly with bacillary products, but no such effects occurred in healthy guinea-pigs injected intraperitoneally. Even in tuberculous animals killed by tuberculin, the death was by slow collapse, without any of the acute respiratory phenomena seen in serum anaphylaxis.

Here was a difference which was hitherto unexplained; since repeated injections of filtered extracts from tubercle bacilli into healthy guinea-pigs failed to sensitize them to any known form of local or general reaction obtainable by tuberculin. On the other hand, dead bacilli were known to do this, but presumably because they created tubercles when injected in sufficient number. The tuberculin effects upon them were nevertheless in all respects similar to those manifested in the virulent inoculated tuberculous animals. It was therefore our first endeavor to sensitize guinea-pigs with various filtered products of tubercle bacilli in such a way as to avoid the production of tubercles.

Methods of preparing bacillary extracts. — The bacilli were grown on ordinary beef broth peptone media containing three per cent glycerin, and were drained off on a suction porcelain filter. They were also washed gently with repeated additions of distilled water until all traces of broth and glycerin were removed. It was thought desirable to avoid any sensitization with products of the bacillus containing

broth constituents which would of themselves sensitize guinea-pigs and thus cause confusion. The extracts were biuret-free when tested for albumoses. Nearly all the sensitizing tuberculo-protein used in this work was prepared at a temperature under 60° C., and was subjected to no chemical influence other than the addition of a little toluol, which was always evaporated prior to animal injections. Sodium chloride was also added to make the extracts isotonic in most of the extracts. The effect of heat upon the sensitizing power of tubercle bacilli preparations is still undergoing study at the laboratory, but is as yet unfinished. (A detailed description of the cultures and preparations used in this work will be found in the Appendix.)

The following experiments were planned to reveal the sensitizing substance if possible by the injection of guinea-pigs with the pure protein extracted by water from tubercle bacilli, and also the pulverized bacillus residue. Others received fractions of the extract precipitated by $(NH_4)_2\,SO_4$. This last was suggested by the work of Gay and Adler,[8] who found the greatest sensitizing element in the globulin fraction of serum protein. Water was used for extraction because it yields a larger amount of protein than N/NaCl.

Part I.

Active sensitization.

Experiment 260B, Aug. 24, 1908:

4 guinea-pigs, average weight 152 grams, received intraperitoneally each .2, .3, 3, .5 cubic centimeter residue 151.
4 guinea-pigs, average weight 146 grams, received intraperitoneally each .1, .2, .1, .1 cubic centimeter W. Ext. 151A.
4 guinea-pigs, average weight 130 grams, received intraperitoneally each .3, .2, .1, .2 cubic centimeter W. Ext. 151C.
6 guinea-pigs, average weight 178 grams, controls.

Sept. 11, 1908. — Seventeenth day. All were injected intraperitoneally with five cubic centimeters of the same extracts and emulsion, except three controls which received ten cubic centimeters of each preparation.

Result: No immediate symptoms suggesting anaphylaxis in any; depression of temperature in four sensitized animals and three controls after eight hours; elevation of temperature in five sensitized, two being residue animals.

Nov. 12, 1908. — Sixty-third day after first test. Average weight, sensitized, three hundred and forty-two, controls three hundred and nine grams. All received five cubic centimeters of same preparations, except three which were given seven cubic centimeters (151C) and three controls N/NaCl solution.

Result: Same as in first test, but less influence on temperature. One control died in twelve hours, collapsed. No symptoms of acute anaphylaxis.

The failure to produce any definite acute symptoms after the first test might have been accounted for by the short period (seventeen days) allowed for the development of sensitiveness or the small amount injected, but the second test indicated that either the doses were too small to exhibit the phenomenon or else the peritoneal injection was ineffective in reaching the seat of sensitiveness. In these animals there

was obviously nothing noted to indicate any difference between the extracts in respect to sensitizing properties.

Forty days after the second test twelve of the above sensitized animals were inoculated subcutaneously with H39, a moderately virulent culture. Twenty-two days later (sixty-two days after the second test) they were all tested by intracardial* or post-orbital (intradural) methods, and found markedly sensitive in the majority of cases, as follows:

2 injected intracardially 1.5 cc. 1 " " 1.0 cc.	Extract emulsion 152. All died at once after a few gasps, without convulsive movements.
2 injected post-orbitally .25 cc.	Extract emulsion 152. One died in 3 minutes with typical anaphylactic symptoms; one coughed, gasped, and revived.
2 injected post-orbitally .5 cc.	Old tuberculin 153. One died 30 seconds convulsive; the other gave no symptoms.
1 injected post-orbitally .3 cc.	Extract emulsion 152. No symptoms.
3 injected post-orbitally .5 cc.	Extract emulsion 152. One died of typical anaphylaxis; the other two reacted positively but revived.

1 suckling young; not tested until later when both mother and young were found fatally sensitive (see Table IX., No. 11).

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*The technic used for the intracardial injections was similar to that described by Morgenroth (Zeitschrift für Hygiene, 1904, xlviii, 177) and Paul A. Lewis (Journ. Exp. Med., 1908, x, 5), in which a needle is passed gently into the right ventricle until the blood appears in a spurt, whereupon the injection of warmed fluid is slowly made. Instead of a syringe, we employed a two cubic centimeter sterile glass pipette provided with a rubber tube connecting with the needle, and a longer piece at the other end for the mouth. By this means, with an assistant to hold the animal, one can blow the fluid more gently and with less danger of lacerating the heart than with a rigid syringe; at least we found this true.

Under the best technic many animals die from hemorrhage into the pericardium, and for that reason we abandoned the method. We also tried intravenous injections through the jugular vein in a few cases, but this method is also rather difficult and uncertain.

The post-orbital injections were made through the cocainized conjunctiva, alongside the eyeball, passing the needle gently through the optic foramen into the cerebrospinal cavity directly under the Pons Varolii.

This method was suggested by the work of Gay and Southard (Journal Med. Research, 1908, xix, 22), and after the technic is acquired by observations on the cadaver, may be performed with surprisingly little inconvenience to the living animals.

These animals revealed but slight tuberculosis, except in the regional lymph glands. The spleen showed beginning involvement; the liver and lungs were free from tubercles; they were well nourished. Those that died with symptoms of respiratory convulsion exhibited markedly distended lungs and sub-epicardial blood extravasations. The significance of this will be referred to later.

To show that the post-orbital injections were not per se the cause of the symptoms, five guinea-pigs were injected with a stronger extract emulsion (No. 152) which was used in subsequent experiments. None of these controls had symptoms, and three had been used for diphtheria antitoxin, which doubtless sensitized them against horse serum.

A few tubercle bacilli extract sensitized guinea-pigs were also injected with broth and normal salt solution without effect.

The work herein described with post-orbital injections relates entirely to guinea-pigs. A few tuberculous rabbits were tried by this method without eliciting symptoms, and one sensitized animal failed to respond to one cubic centimeter post-orbitally.

Sensitization experiment — (continued).

Experiment 260B, Sept. 5, 1908:

4 guinea-pigs, average weight 183 grams, intraperitoneally .3, .2, .3, .5 cubic centimeter 151C "Eu-glob."

4 guinea-pigs, average weight 158 grams, intraperitoneally .3, .2, .3, .5 cubic centimeter 151C residue.

Sept. 21, 1908. — Sixteenth day. One of each series was injected intraperitoneally with ten cubic centimeters W.E. 151A, W.E. 151C "Eu-Gl.," and fifteen cubic centimeters 151C residue. One control received fifteen cubic centimeters W.E. 151C.

Result: No symptoms. Five animals depressed and temperature subnormal in eight hours.

Dec. 16, 1908. Eighty-six days after first test:

6 animals, average weight 419 grams, received five cubic centimeters W.E. 151C intraperitoneally.

4 received 1.5 cubic centimeters intracardially, including two control animals.

Result: The peritoneal animals had no symptoms, but two of the intracardial had respiratory disturbance and cough. Three died in eight to twelve hours, collapsed. Fairly definite evidence of anaphylaxis in two animals; none in intraperitoneal animals. Intracardial injections all successful without pericardial hemorrhage; extract of tubercle bacilli 151C probably too weak to produce marked symptoms.

The general result of our first experiment was to indicate the unsatisfactory character of intraperitoneal tests for demonstrating acute anaphylaxis in tuberculosis, but showed that pure protein extracts of tubercle bacilli were capable of sensitizing guinea-pigs so that intracardial and intradural injections would cause them to react in a characteristic way. The temperature elevation and depression after the peritoneal tests were less marked than in tuberculous animals. The influence of inoculation with living tubercle bacilli on the sensitized animals was not apparent in these experiments, for they were killed after twenty-two days and the control animals proved equally sensitive, as will be seen from the following table (No. 1), which contains some of the animals not inoculated in the preceding experiment.

TABLE NO. I. (Experiment 260 B.)

Sensitization from repeated injections of tubercle bacillus products.

No.	Weight.	Intraperitoneal Injections: Dose and Intervals.			Toxic Injections. Post-orbital.				Autopsy.
					1st.		2d.		
		1st.	2d.	3d.	Interval.	Result.	Interval.	Result.	
13.	185	.3 cc. W.E. 151C.	After 16 days. 16.5 cc. W.E. 151C.	After 86 days. 5 cc. W.E. 148C.	After 29 days. .5 cc. E.E. 152	[1]+++† 5′	. . . . .	. . . . .	Well nourished; epicardial hemorrhages; no tbc.
14.	185	.2 cc. W.E. 151C.	After 16 days. 10 cc. W.E. 151A.	After 86 days. 5 cc. W.E. 148C.	After 29 days. .5 cc. E.E. 152	+++† 8′	. . . . .	. . . . .	Well nourished; epicardial hemorrhages; no tbc.
15.	185	.3 cc. W.E. 151C.	After 16 days. 10 cc. W.E. 151C.	After 86 days. 5 cc. W.E. 148C.	After 29 days. .5 cc. E.E. 152	+	50′ Intracardial. 1 cc. E.E. 152	(Partial success.) +++† 5′	Well nourished; few clots in left thorax; no tbc.
18.	150	.2 cc. Residue 151C.	After 16 days. 10 cc. W.E. 151A.	After 86 days. 5 cc. W.E. 148C.	After 29 days. .5 cc. E.E. 152	+	2⁰ Intracardial. 1 cc. E.E. 152	(Unsuccessful); killed.	Hemothorax; no tbc.
19.	170	.3 cc. Residue 151C.	After 16 days. 17.5 cc. Residue 151C.	After 86 days. 1.5 cc. (Intracardial.)	After 29 days. .5 cc. E.E. 152	+++† 2′	. . . . .	. . . . .	Epicardial hemorrhages; no tbc.
23.	195	10 cc. Residue 151A.	After 63 days. 10 cc. Residue 151 A.	After 50 days. 8 cc. E.E. 152.	After 16 days. .5 cc. E.E. 152	+++	. . . . .	. . . . .	Epicardial hemorrhages; no tbc.
24.	175	5 cc. W.E. 151A.	After 99 days. 5 cc. W.E. 151A.	After 1 day. 5 cc. W.E. 151A.	After 26 days. .5 cc. E.E. 152	+++† 3′	. . . . .	. . . . .	Epicardial hemorrhages; no tbc.

[1] Meaning of signs:
+ Distinct symptoms of anaphylactic sensitiveness.
++ Marked symptoms of anaphylactic sensitiveness.
+++ Very marked symptoms of anaphylactic sensitiveness.
+++† Fatal anaphylaxis.

Symptoms of anaphylaxis. — Something should be said in detail about the symptoms of sensitiveness as exhibited by these animals after the toxic injections. In a perfectly typical instance after a post-orbital injection there was a period of ten to sixty seconds with no symptoms whatever; then urination and defecation took place, followed by uneasiness, scratching of nose, cough and sneezing. This stage was accompanied by respiratory embarrassment, apparent cessation of breathing followed quickly by deep, convulsive respirations, cyanosis of the ears and giddiness. In fatal cases the respirations soon changed to convulsive gasps, the animal fell over, unconscious and kicking. The gasps became shallower, the convulsions ceased, and death occurred in from two to five minutes, the heart still beating after the cessation of breathing.

In the most fulminant cases, the sequence of symptoms was so rapid that only a few gasps presaged death, while in the milder form followed by recovery, the respiratory disturbance was only manifested by deep, slow breathing and uneasiness for several minutes, or only by a few coughs and movements of the mouth as if a disagreeable taste were being experienced. They soon resumed a normal appearance after successful injections.

If the fluid was injected too rapidly or in a quantity over one cubic centimeter, sudden unconsciousness supervened, lasting ten to fifteen seconds, doubtless the effect of pressure in the cerebro-spinal cavity. In other instances a slight giddiness was manifested which quickly passed off. Nonsensitive animals soon resumed their usual activity. A marked characteristic of the anaphylactic reaction appears to be the inspiratory spasm as distinguished from the expiratory act. The death occurs in this phase, and frequently one finds marked emphysema from the distended lungs, while the hemorrhagic extravasations under the visceral pericardium are probably accounted for by the mechanical rupture of the capillaries from violent changes in the thoracic pressure. There were some animals that showed extravasations in the

diaphragm and omentum, but no gastric hemorrhages were noted as found by Gay and Southard [9] in serum anaphylaxis. A careful search was made for gross lesions of the cerebro-spinal region without noting anything abnormal either at or near the site of injection.

Another noteworthy point is that no extravasations occurred in the tuberculous foci of those inoculated animals which were killed by or immediately following the toxic injection with anaphylactic symptoms. The tuberculous animals which survived with or without acute symptoms but died some hours later had the usual focal reactions which are seen in tuberculin poisoning within four to eight hours. Histological examinations were not undertaken in this work which might have revealed more changes, but from naked eye appearances no essential difference was observed in these animals from those sensitized to serum or other proteins.

The symptoms produced by the intracardial injections were less distinct in most cases, in so far as the respiratory disturbance was concerned. The animals either expired at once with a few gasps and convulsions, or gradually lost consciousness and died quietly in ten to fifteen minutes in slower collapse. The manner of death from the intracardial injections resembled that of sensitized rabbits after an intravenous toxic injection. In both, the effect was like a sudden shock and caused immediate unconsciousness.

Having discovered that the post-orbital injection was a reliable method for testing sensitiveness to tubercle bacilli extracts, a variety of experiments were planned to determine the ways by which it could be induced, its relation to progressive inoculation tuberculosis in the guinea-pig, its transmissibility to offspring, and many other questions which naturally arose, notably its relation to immunity and the tuberculin reaction.

Sensitization from a single injection of varying amount: tests at different intervals.

Experiment 274. — The following experiment was intended to cover at least three questions:

(*a*.) Whether the bacterial-free water extract of tubercle bacilli was sufficient to sensitize in a single dose.

(*b*.) Which doses were most sensitizing.

(*c*.) Whether the subcutaneous injection was equal to the peritoneal in sensitizing power.

TABLE NO. II. (Jan. 21, 1909.)

Fifteen guinea-pigs, average weight 233, were injected with filtered water extract 152.

No.	Weight.	Dose, W.E. 152F.	Subcut.	Intrap.	1st Test. Post-orbital. .5 cc. E.E. 152. Feb. 8, 18th Day.	Feb. 12, 22d Day.	Feb. 13, 23d Day.	2d Test. April 1. 47th Day after 1st.	April 2. 48th Day after 1st.	April 6. 52d Day after 1st.	Autopsy.
1.	145	.05 cc.	Subcut.	. . . . .	. . . . .	. . . . .	O	. . . . .	. . . . .	++	
2.	220	.05 "	. . . . .	Intrap.	. . . . .	. . . . .	O	. . . . .	. . . . .	+++ † 3′	Epicardial hemorrhages; normal.
3.	260	.1 "	Subcut.	. . . . .	. . . . .	. . . . .	+	. . . . .	. . . . .	+++	
4.	275	.1 "	. . . . .	Intrap.	O	. . . . .	. . . . .	O Temperature after 6 hours 104.8°.	. . . . .	(3d) O	
5.	270	.15 "	Subcut.	. . . . .	. . . . .	. . . . .	O				
6.	220	.15 "	. . . . .	Intrap.	. . . . .	. . . . .	+ slight.				
7.	250	.25 "	Subcut.	. . . . .	. . . . .	. . . . .	+	. . . . .	.175 cc. + .025 N/Nacl = † 12 hours (intracerebral).	. . . . .	Nothing abnormal.
8.	200	.25 "	. . . . .	Intrap.	++	. . . . .	. . . . .	0.2 cc. (intracerebral) = † 12 hours. Temperature after 6 hours 100.8°.	. . . . .	. . . . .	Nothing abnormal.

9.	240	.5 "	Subcut.	.....	.....	.....	O ?	.....	+++†3′	.....	Epicardial hemorrhages; normal.
10.	240	.5 "	.....	Intrap.	.....	+++†4	.....	.....	.....	.....	Epicardial hemorrhages; normal.
11.	270	1 "	Subcut.	.....	.....	.....	1st right eye = + 2d left eye = O; killed after 15′				
12.	250	1 "	.....	Intrap.	O	.....	.....	.1 cc. + .1 cc. N/Nacl = O (intracerebral). Temperature after 6 hours 102.4°.			
13.	235	1.5 "	Subcut.	.....	.....	.....	+	.....	.....	+	
14.	255	1.5 "	.....	Intrap.	.....	.....	++	.....	.....	+++†3′	Epicardial hemorrhages; normal.
15.	225	2 "	.....	Intrap.	.....	.....	+++†5′	.....	.....	.....	Epicardial hemorrhages; normal.
Anaphylaxis		Positive			1	1	7	0	1	·5	
		Negative			2	0	4	3	1	1	

The animals were all tested post-orbitally with .5 cubic centimeter of extract emulsion 152 after eighteen, twenty-two, and twenty-three days. They were tested with the extract emulsion rather than the filtrate, because it was found that the emulsion acted much stronger than the filtrate.

Result: Nine animals showed more or less symptoms of acute anaphylaxis; two died. Four sensitized subcutaneously had slight symptoms. Five sensitized intraperitoneally reacted severely and two died at once. The greater sensitizing influence of peritoneal injections is readily apparent, but the relation of symptoms to the amount used for sensitization was less evident, as indicated in Nos. 10 and 15. Probably the interval (twenty-three days) was too short for the maximum effect to be exhibited. The surviving animals were tested later and found more sensitive, though of course they had received a further .5 cubic centimeter of the extract. Three of these were injected intracerebrally through the parietal bone, but gave no symptom of acute anaphylaxis by that method. The temperatures were only observed after these latter tests, but were not notably altered.

It is clear from the first tests that small amounts, .1 to .25 cubic centimeter of this water extract, were capable of sensitizing guinea-pigs, while .5 cubic centimeter sufficed to produce fatal sensitiveness in twenty-two days without the presence of tubercles or of living or dead tubercle bacilli in the tissues.

No attempt was made in these tests to determine the minimal quantity of the emulsion necessary to produce symptoms from the post-orbital injections.

In Table II. (a) are shown some tests made with larger amounts and also at different times after the sensitizing injection. Individual variations are to be noted in the sensitiveness.

TABLE II. (a). (Experiment 278, May 17, 1909.)

Sensitization from a single injection of the same amount: tests at different intervals.

No.	Weight.	Sensitized Intraperitoneally.	1st Test.			2d Test.			Autopsy.
			Interval.	Dose.	Result.	Interval.	Dose.	Result.	
5.	360	1.5 cc. W.E. 154	24 days.	.5 cc. E.E. 154X	++	.....	.....	.....	(Inoculated later).
7.	425	1.5 " " "	57 "	.75 " " "	+++† 5	.....	.....	.....	Epicardial hemorrhages.
9.	250	1.5 " " "	24 "	.75 " " "	O	57 days.	.75 cc.	+++† 2′	Extensive epicardial hemorrhages.
30.	280	1.5 " " "	31 "	.75 " " "	+	1 hour.	.75 cc. (left eye).	O	Killed; nothing abnormal noted.
31.	290	1.5 " " "	49 "	.75 " " "	+++† 5′	.....	.....	.....	Extensive epicardial hemorrhages.
34.	510	1.5 " " "	19 "	1 " " "	+	18 days.	.75 cc.	O	(Inoculated later.)
37.	515	1.5 " " "	37 "	.75 " " "	O	(Poor nutrition.)			Died enteritis (?)
39.	320	1.5 " " "	10 "	1 " " "	O	.....			" "

Result: Five out of seven animals reacted. Those tested after the longest interval had the most sensitiveness. Variation of the toxic dose is of less moment than the time interval, and repetition after a long interval discloses sensitiveness as in Table II.

The guinea-pigs used for controls in testing for anaphylaxis by post-orbital and intracerebral injections were later tested for sensitiveness acquired by this route. It was somewhat unexpectedly found less than that from the peritoneal injections. The number was, however, not large enough to warrant any definite decision. Theoretically it was to be expected that these animals would be rendered highly sensitive by injections so near the supposed center of greatest sensitiveness. Possibly the time for incubation should be longer, and this was undetermined in our experiments. Examples are to be seen in the accompanying table.

TABLE II. b. (Experiment 260D, Jan. 4, 1909.)

Sensitization from post-orbital injections.

Sensitized, Post-orbital Right Eye.			1st Test.			2d Test.			Autopsy.
No.	Weight.	Dose.	Interval.	Post-orbital.	Result.	Interval.	Post-orbital.	Result.	
4	365	1 cc. E.E. 152	16 days.	.5 cc. E.E. 152	+ ?	47 days.	.5 cc. E.E. 152	+++	Nothing abnormal.
14	365	1 " " "	92 "	.5 " " "	++	32 "	.5 " " "	+	" "
17	205	.25 " " "	42 "	.5 " " "	+ (Killed.)	. . . . .	. . . . .	. . . . .	Small epicardial hemorrhages; otherwise normal.
5. (Sensitive to horse serum also)	15/1/09 370	.5 " " "	41 "	.5 " " "	+++ † 8′	. . . . .	. . . . .	. . . . .	Epicardial and diaphragmatic hemorrhages. No tbc.
20	355	.5 " " "	22 "	.5 " " "	O	77 days.	.2 cc. E.E. 152	O	
21	180	.5 " " "	22 "	.5 " " "	+ ?	65 "	.5 " " "	+	
25	175	.5 " " "	22 "	.5 " " "	O	65 "	.5 " " "	O	
Ex. 273. 2-A . .	6/2/09 160	.3 " " "	69 "	Intracerebral, .05 cc. + 1.95 N/Nacl.	O	93 "	.5 " " "	+++ † 10′	Nothing abnormal.
3-A	2/3/09 270	.5 " " "	45 "	Intracerebral, .05 cc. + 1.95 N/Nacl.	O	93 "	.5 " " "	+	Killed; well nourished; normal.
4-A	8/3/09 220	.5 " " "	39 "	Post-orbital, .5 cc. E.E. 152	++	93 "	.5 " " "	O	
2	625	.5 " " "	74 "	.75 cc. W.E. 154 Bx.	O	. . . . .	. . . . .	. . . . .	Died; nothing abnormal.
3	610	1 " " "	74 "	.75 cc. W.E. 154 Bx.	O	4 months.	.75 cc. E.E. 152	++	Killed; slight epicardial hemorrhages.

Result: Only half of the animals were sensitive at the first test. The individual difference was considerable.

Intracerebral sensitization. — A few animals that survived injections directly into the brain were likewise found less sensitive for the most part to post-orbital injections than those peritoneally sensitized.

TABLE II. C. (Experiment 273 A.)

No.	Weight.	Sensitized Intracerebrally.	1st Test.			2d Test.			Autopsy.
			Interval.	Post-orbital.	Result.	Interval.	Post-orbital.	Result.	
1 Bx .	250	.15 cc. E.E. 152	16 days.	.5 cc. E.E. 152	++	. . . . .	. . . . .	. . . . .	Killed; slight epicardial hemorrhages.
7C . .	250	.15 cc. E.E. 152	19 "	.5 cc. E.E. 152	O	78 days.	.5 cc. E.E. 154X	+++†3′	Marked epicardial hemorrhages.
8D . .	215	.15 cc. E.E. 152	28 "	.5 cc. E.E. 154X	O	6 "	.5 cc. E.E. 154X	O	(Still living.)
Ex. 261. 35 . .	585	.15 cc. E.E. 152	6½ mos.	.75 cc. E.E. 158	+	. . . . .	. . . . .	. . . . .	" "

Result: Two of the four showed slight symptoms on the first test. Note fatal result on second test, No. 7C.

Besredka and Steinhardt[10] failed to sensitize with horse serum by intracerebral injections. This would be explainable if the antibody were chiefly produced in other tissues, or if owing to its concentration the antigen prevented the formation of antibody in the nerve tissues. The former explanation appears more plausible since sensitized animals may recover from a toxic injection into the brain, yet die from anaphylaxis after a subsequent test. Further experiments to determine the dose necessary to sensitize may bear on this point.

Anaphylactic sensitiveness of tuberculous guinea-pigs. — In order to determine whether the actual disease, tuberculosis, confers this form of sensitiveness on animals, the following experiment was carried out:

Experiment 275, Jan. 21, 1909. — Fifteen guinea-pigs, average weight two hundred and eighteen grams, were inoculated with .125 cubic centimeter emulsion of H39 serum culture, 30–XII–08, subcutaneously, in the right groin. Smears of the emulsion show five to ten tubercle bacilli to a field. The animals were tested in succession, post-orbitally, with .5 cubic centimeter extract emulsion 152, as indicated in Table III. and then killed to note the extent of the disease.

TABLE III.

No.	21-1-09. Weight.	18th Day, 8-2-09.		22d Day, 12-2-09.		23d Day, 13-2-09.		34th Day, 24-2-09.		42d Day, 4-3-09.		53d Day, 15-3-09.		54th Day, 16-3-09.	99th Day, 1-4-09.
		Weight.	Test.	Weight.	Test.	Weight.	Test.	Weight.	Test.	Weight.	Test.	Weight.	Test.	Test.	Test.
1.	165	190	O; killed.												
2.	235	. . . . .	. . . . .	305	O; killed.										
3.	245	. . . . .	. . . . .	310	+										
4.	265	330	O												
5.	205	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O								
6.	205	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O								
7.	210	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O; killed.								
8.	210	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O								
9.	280	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	330	+; killed.						
10.	230	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	295	+ + + † 4′						
11.	160	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O; † in night.	
12.	260	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	O; depressed.	

13.	110	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	350; O; † in night.
14.	280	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	390	O; depressed.	. . . . .	. . . . .	. . . . .	. . . . .		
15.	215	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	350	O; † in night.				
Extent of disease.		No. 1. Tuberculous; right inguinal nodes beginning caseation; otherwise normal.		No. 2. Cold abscess site inoculation; caseous right inguinal nodes; enlarged iliac nodes; spleen enlarged.		No. 7. Same as No. 2 but caseated iliac nodes.		Nos. 9 and 10. Disease has reached spleen which contains nodules; liver free; nodes caseous.		. . . . .		No. 15. Advanced chronic tuberculosis all organs.		No. 11. Advanced chronic tuberculosis all organs.		No. 13. Emaciated; advanced chronic tuberculosis.

Result: Only three of the animals exhibited anaphylactic symptoms when tested. It is evident that inoculation tuberculosis produces but little sensitiveness, or, what is more probable, that the reacting power is continually exhausted by the progress of the disease. It is noteworthy that the animals last tested died of tuberculin collapse, though they had no immediate symptoms following the test injection. The latter presented the usual signs of bloody extravasations in the spleen and liver, indicative of the focal reaction.

Sensitiveness of tuberculous guinea-pigs; repeated inoculations.

Experiment 266A. — Ten of these animals had been repeatedly inoculated subcutaneously with emulsions of lymph nodes from tuberculous guinea-pigs; ten others were once inoculated with pus from cold abscesses, and five were controls. The entire lot were subsequently inoculated (Dec. 7, 1908), with an emulsion containing one tubercle bacillus to two fields, .1 cubic centimeter subcutaneously to each animal, H37 serum culture 25–XI–08. Twenty-one animals were tested after forty-nine and fifty days as follows:

TABLE IV. (Experiment 266A.)

No.	Lymph Node Emulsion.	Cold Abscess Pus.	49th Day after Virulent Inoculation.		50th Day after Virulent Inoculation.	
			Test.	Symptoms of Anaphylaxis.	Test.	Symptoms of Anaphylaxis.
1.	Lymph node emulsion.	. . . . .	.5 cc. Ext. Emul. 152	O	. . . . .	(Tested May 12.) .5 cc. Ext. Emul. 152 = O.
2.	" " "	. . . . .	. . . . .	. . . . .	.5 cc. Ext. Emul. 152	O; killed 8 P.M.
3.	" " "	(Suckling 2 young.)	. . . . .	. . . . .	—	
4.	" " "	. . . . .	. . . . .	. . . . .	.5 cc. Ext. Emul. 152	O
5.	" " "	. . . . .	. . . . .	. . . . .	.5 " " " "	+; killed 8 P.M.
6.	. . . . .	Cold Abscess Pus. (Suckling 2 young.)	.5 cc. O.T. 153	+?; † 8 hours.	—	
7.	. . . . .	† XII–22, enteritis.	—	—	—	
8.	. . . . .	(Suckling 2 young.)	. . . . .	. . . . .	.5 cc. Ext. Emul. 152	+++† 15′
9.	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " " "	++; killed 8 P.M.
10.	. . . . .	. . . . .	. . . . .	. . . . .	.5 " " " "	O
11.	Lymph node emulsion.	. . . . .	.25 cc. O.T. 153 +, .75 N/Nacl.	O		
12.	" " "	. . . . .	. . . . .	. . . . .	.5 " " " "	(Tested May 12.) .5 cc. Ext. Emul. 152 = O.
13.	" " "	. . . . .	. . . . .	. . . . .	.5 " " " "	O; killed 8 P.M.
14.	" " "	(Suckling 2 young.)	—	—	—	
15.	" " "	. . . . .	. . . . .	. . . . .	.5 cc. O.T. 153	+?; † in night.

TABLE IV. (Experiment 266A.) — *Continued.*

No.	Lymph Node Emulsion.	Cold Abscess Pus.	49th Day after Virulent Inoculation.		50th Day after Virulent Inoculation.	
			Test.	Symptoms of Anaphylaxis.	Test.	Symptoms of Anaphylaxis.
16.	. . . . .	Cold Abscess Pus. (Suckling 2 young.)	. . . . .	. . . . .	.5 cc. Ext. Emul. 152	+ + + † 5′
17.	. . . . .	. . . . .	1 cc. Ext. Emul. 152	+	—	
18.	. . . . .	. . . . .	. . . . .	. . . . .	.3 cc. O.T.153,+.2 N/Nacl.	+ ? ; † in night.
19.	. . . . .	. . . . .	. . . . .	. . . . .	.5 " Ext. Emul. 152	O
20.	. . . . .	. . . . .	. . . . .	. . . . .	.3 " O.T.153,+.2N/Nacl.	O; killed 8 P.M.
21.	Control.	(Pregnant.)	. . . . .	. . . . .	(Tested April 12) .2 cc. Ext. Emul. 152	O; † in night.
22.	"	. . . . .	.5 cc. Ext. Emul. 152	+ ?	—	
23.	"	. . . . .	.5 " " " "	O; † 4 hours.	—	
24.	"	. . . . .	.5 " W.E. 152	+ ?; † 6 hours.	—	
25.	"	. . . . .	.5 " Ext. Emul. 152	+ + + —	Killed.	Generalized caseous tub.

Result: Five animals had distinct symptoms of acute anaphylaxis which was fatal in two. Five had doubtful reactions. Four died of tuberculin collapse later. Five were killed in a moribund state. Old tuberculin failed to produce anaphylaxis. No relation of the severity of the anaphylactic symptoms to the extent of disease can be inferred. Note that No. 25, which had marked symptoms, was extensively diseased, while No. 16 had only caseated nodes at the site of inoculation, yet died promptly of anaphylaxis. Note also the larger number of positive reactions in the abscess-pus animals.

The surviving animals were killed by puncture of the medulla, and most of these were dying from tuberculin collapse. The tuberculosis had extended to a varying degree in the different sets of animals; the controls mostly had caseated spleen and liver and some infiltration of lungs; the lymph-node animals showed some enlargement of the spleen and beginning caseation, but the abscess-pus animals showed barely anything beyond caseated inguinal, iliac and retroperitoneal nodes.

The greater sensitiveness of the abscess-pus animals is apparent, yet its significance less so. The explanation might be offered that as they had acquired resistance from the first inoculation of abscess pus, one expression of this was their greater sensitiveness to this test.

The above experiment was repeated with practically the same results in the following:

Repeated inoculations.

Experiment 276. — Ten guinea-pigs were inoculated first with lymph node emulsion and abscess pus. On June 28, 1909, together with six controls, they received .1 cubic centimeter emulsion H37 culture 27–VI–09 subcutaneously in the right groin. Ten were tested after fifty-five days as shown in the table:

TABLE V.

Fifty-five days after virulent inoculation.

No.	Cold Abscess Pus.	Test.	Result.
11 ..	Cold abscess pus.	.5 cc. Ext. Emul. 158	O
12 ..	" " "	.75 " " " "	O
13 ..	" " "	.75 " " " "	O
15 ..	" " "	.75 " " " "	+ + + † 2′
16 ..	Lymph node emulsion.	.75 " " " "	O
18 ..	" " "	.75 " " " "	O
20 ..	" " "	.75 " " " "	O
26 ..	Control.	.75 " " " "	O
27 ..	"	.75 " " " "	O
29 ..	"	.75 " " " "	O

Result: Only one pig sensitive, and that one of the abscess-pus animals. All the others were sacrificed on the same day but revealed little differences in the extent of tuberculosis present. The disease had reached the spleen in but few instances. No. 15 had no different lesions from the majority, though markedly susceptible.

Bovine bacillus inoculations. — In addition to the preceding experiments a few other tests were made on tuberculous guinea-pigs with extract emulsion 152.

Two guinea-pigs inoculated with bovine tubercle bacilli "B_1" were tested after fifty-two and one hundred and thirteen days, respectively. No symptoms of anaphylaxis followed, but both died of tuberculin collapse and revealed generalized caseous tubercle.

One guinea-pig tested ten months after inoculation (7–VII–08) with .00025 R1 weak virulent culture followed by recovery (?) showed very slight symptoms.

Summary: Inoculation tuberculosis gives rise to an

uncertain degree of anaphylactic sensitiveness without recognizable relation to the amount of disease in the animal. In the advanced stages the sensitiveness is abolished in most cases, so far as acute reaction is concerned. Repeated inoculation appeared to increase sensitiveness in guinea-pigs.

The relation of tuberculin focal and general reactions to anaphylactic symptoms is discussed on page 44.

Inheritance of sensitiveness: *a*. Maternal tuberculosis. — The transmission from mother to offspring of anaphylactic sensitiveness seemed to be very probable from analogy with serum experiments. This had been already suggested by Rosenau and Anderson, but no demonstration has, to my knowledge, been made of such in connection with tuberculosis.

The following observations were made on the offspring of female guinea-pigs inoculated while (*a*) suckling young, (*b*) during pregnancy, and (*c*) males which were afterwards mated with healthy females:

Experiment 273, Jan. 21, 1909. — (*a*.) Two females which were suckling five young born 12–1–09 were inoculated in the right side of the neck subcutaneously with a dilute emulsion of H39 culture, .25 cubic centimeter each.

(*b*.) Five pregnant females, ditto, right axilla.

(*c*.) Two males, ditto, right inguinal.

Especial care was taken to cover the needle puncture with collodium to prevent infection of the young from outside contact. The young were tested at different times, after the inoculation of the mother pigs, as shown by the table, and then separated from them. Some were tested intracerebrally because of the failure to produce anaphylaxis by the post-orbital route.*

* The intracerebral method employed was that described by von Lingelsheim.[11] The animal when under the first stage of ether anesthesia was trephined through the left parietal bone with a small dental drill. Two-tenths cubic centimeter of the fluid was slowly injected at a depth of five millimeters. Previous experiences had shown the futility of intraperitoneal injections as a test of inherited sensitiveness to tuberculin.

TABLE VI. (a).

Suckling Females Inoculated.	Weight. 21-1-09.	Offspring. No.	Weight.	Age and Test.	Result.	Test of Mother.	Result.
No. 1 . . .	590	4A.	220	46th day, postorbitally .5 cc. Ext. Emul. 152.	O		
		5A.	335	67th day, intracerebrally .2 cc. Ext. Emul. 152.	O; † 6 hours.	14/4/09. 1 cc. intraperitoneally Ext. Emul. 152.	O; collapse; † 8 hours.
		6A.	350	79th day, .15 cc. Ext. Emul. 152 + .05 N/Nacl.	O; † 24 hours.		
No. 2 . . .	715	2A.	160	16th day, postorbitally .3 cc. Ext. Emul. 152.	O	14/4/09. 1 cc. intraperitoneally Ext. Emul. 152.	O; collapse; † 8 hours.
		3A.	270	40th day, .5 cc. Ext. Emul. 152.	O		
Healthy (control).	. . .	5	350	Intracerebrally .2 cc. Ext. Emul. 152.	O	. . . . .	(See Table VII. (b) for other control injections.)

Result: Generally considered there was no evidence of transmitted sensitiveness through the milk. The fatal result in two pigs injected intracerebrally suggests greater sensitiveness than controls, but not manifested by acute anaphylaxis.

TABLE VII. (B).

Pregnant Females Inoculated.	Litter.	Offspring.	Weight.	Age and Test.	Result.	Test of Mother.	Result.
27–II–09.							
No. 5	(2) 19–II.	8D.	215	55 days, .15 cc. Ext. Emul. 152 + 05 N/Nacl intracerebrally.	O	No. 5, † ?	
		9D.	. . . . .	83 days, .5 cc. Ext. Emul. 152 post-orbitally.	O		
No. 6	(2) 17–II.	7C.	210	54 days, .15 cc, Ext. Emul. 152 + .05 N/Nacl intracerebrally.	O; (weak but revived).	No. 6, 1–V, .5 cc. Ext. Emul. 152 post-orbitally.	O; † 6 hours.
		8C.	† 8–III.				
No. 7	(2) 7–III.	10E.	180	39 days, .15 cc. Ext. Emul. 152 + .05 cc. N/Nacl intracerebrally.	O; † in night.		
		11E.	200	.5 cc. Ext. Emul. 152 post-orbitally.	O; (killed in 10').	No. 7, 1–V, .5 cc. Ext. Emul. 152.	O; † 6 hours.
No. 8	(Aborted.)						
No. 9	(3) 2–III.	1B.	145	34 days, .4 cc. Ext. Emul. 152 post-orbitally.	O; (weak but revived).	No. 9, 14–V, 1 cc. Ext. Emul. 154 Res. intraperitoneally.	O; † 8 hours.
		2B.	155	41 days, .15 cc. Ext. Emul. 152 + .05 cc. N/Nacl intracerebrally.	† 4 hours.		
	Control, healthy.	1 Bx.	250	.15 cc. Ext. Emul. 152 + .05 N/Nacl intracerebrally.	O; (weak but revived).		
No. 10 . . .	(2) 2–II.	3B.	135	24 days, .4 cc. Ext. Emul. 152 post-orbitally.	O		
		4B.	120	31 days, .5 cc. Ext. Emul. 152 post-orbitally.	O	No. 10, 4–V, .1 cc. W.E. 154 intraperitoneally.	O; † 8 hours.

TABLE VII. (b) continued.

(*Miscellaneous.*)

Tuberculous Females.	Litter.	Offspring. No.	Weight.	Age and Test.	Result.	Test of Mother.	Result.
263. No. 10. .	(2) 18-IV-08.	2	625	11 months, .5 cc. Ext. Emul. 152 post-orbitally.	O	No. 10, † 4-VI (no signs of tuberculosis; healed?).	
		3	610	1 cc. Ext. Emul. 152 post-orbitally.	+ ?		
266A. No. 14.	(1) 20-II.	4	120	34 days, .4 cc. Ext. Emul. 152 post-orbitally.	O	76 days.	
No. 3	(2) 25-II.	16F.	280	50 days, .15 cc. Ext. Emul. 152 + .05 N/Nacl intracerebrally.	O; † 6-8 hours.	No. 3, .5 cc. 1st Res. 152 post-orbitally.	O
		3X.	. . . . .	76 days, .5 cc. Ext. Emul. 152 post-orbitally.	O		
266. No. 14 .	(1) 20-II.	5	195	16 days, post-orbitally .4 cc. Ext. Emul. 152.	O		
	(2) -VI(?).	14B.	230	60 days?, .6 cc. Ext. Emul. 152 post-orbitally.	O		
		14C.	180	.6 cc. Ext. Emul. 152 post-orbitally.	O		
266A. No. 14.	(1) 2-II.	13	210	.15 cc. Ext. Emul. 152 intracerebrally.	O; † 24 hours.	1-IV-09. .5 cc. 1st Res. 152 post-orbitally.	O; † 8 hours.
270. No. 8. .	(2) 3-III.	8A.	. . . . .	† Marasmus.	O	(No. 8, killed; tuberculin, O.T. 153.)	
		8B.	150	55 days, .5 cc. Ext. Emul. 152 post-orbitally.			
No. 11	(2) 19-III.	11A.	310	4 months, .5 cc. 154X B.E. post-orbitally.	O		
		11B.	150	28 days, .4 cc. Ext. Emul. 152 post-orbitally.	O		

TABLE VII. B, continued. (Experiment 273 A–B.)

Control intracerebral tests.

Healthy.	Control.	Weight.	Test.	Anaphylaxis.
Healthy.	No. 8.	240	.2 cc. Ext. Emul. 152.	O; (killed 24 hours).
"	" 14.	270	.15 cc. Ext. Emul. 152 + .05 cc. N/NaCl.	O; † 7 days.
"	" 15.	160	.15 cc. Ext. Emul. 152 + .05 cc. N/NaCl.	O; † 28 hours.
"	(Had been used for diphtheria antitoxin test.) " 6.	355	.2 cc. Ext. Emul. 152.	O; † 6 "
"	" 7.	305	.2 " " " 152.	O; † 8 "
"	" 8X.	215	.2 " " " 152.	O; † 8 "
"	" 10.	290	.2 " " " 154F.	O; killed 19-IV-09.
"	" 11.	305	.2 " B.F. Tuberculin.	O; " "
"	" 9.	250	31-III-09. .1 cc. Ext. Emul. 152 + .1 cc. N/NaCl.	O; " 27-IV-09.
"	" 12.	290	.175 cc. Ext. Emul. + .025 N/NaCl.	O; † 6 days.
"	" 13.	185	.150 cc. Ext. Emul. 152 + .05 N/NaCl.	O; † (?) "
Tuberculous Inoculated.	" 8.	270	.2 cc. Ext. Emul. 152.	O; † 8 hours.
Tuberculous Inoculated.	" 6.	330	.2 " " " 152.	O; † 10 "

Result: There was no instance of acute anaphylaxis among the young guinea-pigs from tuberculous mothers, including females inoculated during the pregnancy and others actively diseased before pregnancy. By the intracerebral test there is some apparent susceptibility as compared with control pigs from healthy mothers.

TABLE VIII. (c).

Healthy Females mated with Inoculated Males.	Litter.	Offspring.	Weight.	Age and Test.	Result.	Test of Mothers.	Result.
No. 13 ..	(3) 30–IV.		All dead.				
		14D.	250	60 days (?), .5 cc. E.E. 154X post-orbitally.	O		
No. 14...	(2)	14E.	260	.5 cc. E.E. 154 X post-orbitally.	O	No. 14, .75 cc. E.E. 154 X post-orbitally.	O

Result: Negative.

The unreliability of the intracerebral test is so great that not much value can be claimed for the results in the foregoing table (VII. B). Furthermore, the use of ether anesthesia might mask the manifestations of acute anaphylaxis. As further evidence of the doubtful value of this method, the results in artificially sensitized animals (Table IX.) should be noted.

Since actively tuberculous guinea-pigs are seldom found to give acute anaphylactic symptoms, it was not to be expected that their offspring would show it. Unfortunately only a few of the inoculated females in the preceding experiment were tested for acute anaphylaxis.

The following experiment, however, illustrates a marked transmitted anaphylactic sensitiveness from the maternal side, when the pregnant females are simply sensitized with tubercle bacillary extract, but not inoculated.

TABLE IX.

Inheritance of sensitiveness: maternal sensitization.

Pregnant Females.	Weight.	Sensitized.	Offspring.		Weight.	Test.	Result.	Test of Mother.	Result.
Exp. 273A. No. 11.	430	13-III-09. 2.5 cc. W.E. 152F. intraperitoneally.	(1) 11A.		135	1-V-09. .3 cc. Ext. Emul. 152 post-orbitally.	+++	1-V-09. 76 days after sensitization, .5 cc. Ext. Emul 152 post-orbitally.	+++† 2′
No. 12	450	2 cc. W.E. 152F.	(2) Both † premature.						
Exp. 260B. No. 11 (inoculated later).	. . .	12-XI-08. 7 cc. W.E. 151C intraperitoneally.	(2) 28-XII-09.	8	110	7-I-09. .25 cc. Ext. Emul. 152.	+++† 1′	13-I-09. 62 days after sensitization, 1.5 cc. Ext. Emul. 152 intracardially.	† at once.
				9	105	.25 " " " "	+++† 1′		
270D. No. 22 . .	405	15-I-09. Ext. Emul. 1 cc. 152 intracardially.	(2) 13-III.	22A	165	13-IV-09. .15 cc. Ext. Emul. 152 + .05 cc. N/Nacl intracerebrally.	(Unsuccessful; killed).	13-IV-09. (530) .2 cc. Ext. Emul. 152 intracerebrally.	O; †Shours.
				22B	185	.15 cc. Ext. Emul. 152 + .05 cc. N/Nacl intracerebrally.	O; † 8 hours.		
260B. No. 5 . .	375	12-II-08. 5 cc. W.E. 151A intraperitoneally.	13-I-09. (1) No. 16.		270	23-II-09. .5 cc. Ext. Emul. 152 post-orbitally.	+++† 2′		
			(2d litter.) 11-III-09. (2)	11	160	10-IV-09. .4 cc. Ext. Emul. 152 post-orbitally.	+++† 2′	10-IV-09. (470) .5 cc. Ext. Emul. 152 post-orbitally.	+++† 3′
				12	150	.4 cc. Ext. Emul. 152 post-orbitally.	+++† 1′		

TABLE IX. — *Continued.*

Inheritance of sensitiveness: maternal sensitization.

Pregnant Females.	Weight.	Sensitized.	Offspring.		Weight.	Test.	Result.	Test of Mother.	Result.
260D. No. 14 . . .	365	8-I-09. 1 cc. Ext. Emul. 152 post-orbitally.	7-III-09. (2)	9d	200	10-IV-09. .4 cc. Ext. Emul. 152 post-orbitally.	+++	10-IV-09. (430) .5 cc. Ext. Emul. 152 post-orbitally.	++
				10d	190	.4 cc. Ext. Emul. 152 post-orbitally.	+++		
273A. No. 1 "B.F."	490	14-IV-09. 1 cc. Ext. Emul. 152 intraperitoneally.	16-III-09. (2)	1A	. . .	12-V-09. .5 cc. Ext. Emul. 152.	+++ † 2′	12-V-09. .5 cc. Ext. Emul. 152 post-orbitally.	+
				1B	. . .	† (soon after birth).			

Results: Ten offspring and six mothers all gave positive symptoms of acute anaphylaxis. The striking uniformity exhibited in the anaphylaxis transmitted from the maternal side as well as the severity of the symptoms is in marked contrast to the previous experiments with the young of inoculated females. The inference is therefore justified that the causative agent of this reaction is transmissible through the placental blood as in serum anaphylaxis.

The reason suggested for the absence of symptoms in the test for anaphylaxis in tuberculous animals applies to their offspring. The constant absorption from foci of disease probably keeps most of the animals in a condition of so-called anti-anaphylaxis, or prevents the development of the antibody in sufficient amount to be transmitted to the offspring.

It is nevertheless probable that if more delicate methods were known for detecting inherited qualities, a specific influence of some sort could be demonstrated in the offspring of tuberculous animals as well as in those sensitized according to the method shown in Table IX. Whether such an influence might take the form of an inherited predisposition to the disease or not is a problem of considerable difficulty from the standpoint of experiments on laboratory animals.

Anti-anaphylaxis. — The parallelism of tuberculo-protein sensitization to other forms is further shown by the failure to elicit a second reaction, or weakening of the same, after a repetition of the injection within a short time. The refractory condition was not invariably produced by the first dose but seemed quite distinct.

TABLE X.

Sensitized Guinea-pig.	1st Test.	Result.	2d Test.	Result.	Autopsy.
260B. No. 15.	Post-orbital injection.	+ (?)	After 50′ intracardially.	+++ † 15′	Epicardial hemorrhages.
No. 28.	" " "	++	" 26′ "	O	
260D. No. 2.	" " "	+	" 32 hours intracardially	O	
269. No. 4.	" " "	+++	" 32 " "	++	
277. No. 1.	" " "	+++	" 32 " "	+ ?	
No. 3.	" " "	+++	" 32 " "	O	
No. 5.	" " "	+	" 32 " "	O	
274. No. 2.	" " "	+	" 15′ intracardially.	O (?)	
278. No. 30.	" " "	+	" 1 hour intracardially.	O	

Result: Seven of nine tests were negative where the previous test had been positive; two simply showed a diminished response.

Occasionally the second injection first produced the symptoms of anaphylaxis, more especially when given intracardially, as seen below:

TABLE XI.

Sensitized Guinea-pig.	1st Test. Post orbital: Reaction.	2d Test.
260B. No. 27....	O	After 4 hours intracardially. + + † 15′
275. No. 2....	O	After 40 minutes intracardially. + ?
277. No. 2....	O	After 25 minutes post-orbitally. + ?

Of course the question of the connection between the last noted symptoms and anaphylaxis may be raised, since the sensitive animals die or become very weak without respiratory spasm after intracardial injection.

Effect on temperature. — It was of interest to observe the effect on temperature of a toxic injection followed by

non-fatal symptoms of anaphylaxis in sensitized animals, in comparison with tuberculous animals. Some mention is made in the experiment first detailed of the effect on temperature after intraperitoneal and intracardial tests, but the following relates to post-orbital tests followed by positive symptoms:

TABLE XII.

Sensitized Guinea-pigs.	Test.	Result.	Temperature after 7 Hours.
No. 1	2d	+ ?	99° F.
277. No. 2	1st	+	98.2
No. 3	1st	+ + +	100.2
No. 4	2d	+	96.4
No. 5	1st	+	96.2
273. No. 3A	3d	+	104.8
270. No. 11B	2d	+	102.4

Result: Depression of temperature is evident in some and elevation in others, as in tuberculous animals. Some of the animals included in the above table corresponded to inoculated tuberculous guinea-pigs because they had previously received dead bacilli in the former tests. As the temperature of these animals is readily affected by excitement and trauma, no especial significance attaches to the variations.

Effect of different bacillary preparations in sensitization. — It was of interest to determine whether the washed residue of extracted bacilli was still capable of sensitizing animals after removal of the water-soluble substances. To this end three guinea-pigs were injected intraperitoneally with two cubic centimeters W.E. 154, together with four which received the Residue 154 suspended in an equal amount of normal saline.

TABLE XIII. (Experiment 277.)

No.	Weight.	Sensitized Water Extract. 26-III-09.	Post-orbital Test.		Result.	Autopsy.
			Interval.	Dose.		
1.	420	2 cc. W.E. 154	31 days.	.5 cc. W.E. 152	+++	No record.
2.	405	2 " " "	32 "	.5 " " "	+	Killed 28-IV; nothing abnormal.
3.	390	2 " " "	32 "	.5 " " "	++	† 29-VIII; malnutrition; nothing abnormal.
4.	475	Residue. 2 cc. Res. 154	31 "	.5 " " "	O	Killed 28-IV; encapsulated caseous masses in omentum.
5.	400	2 " " "	32 "	.5 " " "	+	Killed 28-IV; same as No. 4.
6.	375	2 " " "	.....	.....	.....	Died 12-IV; same as No. 4.
7.	400	1.5 " " "	.....	.....	.....	Died 26-IV; plastic peritonitis from tubercle bacilli residue.

Result: Indications of greater sensitiveness in the extract than in the residue animals, yet some effect is produced by the residue, which contains very little soluble protein.

The experiment was repeated later on six guinea-pigs, using one cubic centimeter of each preparation of 154, as in Experiment 277. Only one "Residue" pig was sensitive after thirty-one days. The others died and the three "Extract" pigs failing to react, probably because of poor condition, the experiment was of little value.

It is evident that enough protein was set free in the animals from the residue of tubercle bacilli to sensitize them to a slight extent. The following experiment confirms this, and was made with a residue emulsion of approximately the same bulk per weight as the extract taken from the bacilli, which were first pulverized.

TABLE XIV. (Experiment 288, 16–11–09.)

Sensitization with "T.R." (tuberculin residue). Ten guinea-pigs received 2.5 cubic centimeters intraperitoneally T.R. emulsion No. 158.

No.	Weight.	Interval.	Post-orbital.	Result.	Autopsy.
34.	320	. . . . .	. . . . .	. . . . .	Died 17–11–09; nothing abnormal.
35.	300	50 days.	.75 cc. E.E. 161	+	Reserved.
36.	365	50 "	.75 " " "	+	"
37.	280	48 "	.75 " " "	+++† 2½′	Marked epicardial hemorrhages and inflation of lungs; no tuberculosis.
38.	370	48 "	.75 " " "	+++† 3′	Marked epicardial hemorrhages and inflation of lungs; no tuberculosis.
39.	270	50 "	.75 " " "	+++† 2′	Marked epicardial hemorrhages and inflation of lungs; no tuberculosis; spleen adherent to peritoneal wall.
40.	290	. . . . .	. . . . .	. . . . .	Reserved.
41.	310	48 days.	.75 cc. E.E. 161	++	Killed; no tuberculosis; nothing abnormal.
42.	(pregnant) 410	. . . . .	. . . . .	. . . . .	Reserved.
43.	340	. . . . .	. . . . .	. . . . .	"

Result: All of the six animals tested were found sensitive and three fatally so. It is probable that the crushed residue which forms the T.R. acts better in sensitizing than the intact bacilli because more thoroughly absorbed.

Effect of different bacillary preparations for toxic injections. — It has been noted that a distinct difference was observed between the toxicity of filtered and unfiltered extracts of tubercle bacilli. The former caused much more reaction than the latter, and the filtration through the bougie must keep back something which in the form of an emulsion is more active.

The following experiments are illustrative:

Experiment	266A,	No.	24.	Injected on	49th day	.5 cc.	W.E. 152F = O
"	"	"	25.	"	" " "	.5 "	E.E. 152 = +++
"	277	"	2.	"	" 32d "	.5 "	W.E. 154 = O
				"	after 25 min.	.5 "	E.E. 152 = +
"	278	"	29.	"	on 49th day	.75 "	W.E. 154X = O
"	"	"	31.	"	" " "	.75 "	E.E. 154X = +++† 5′

The reason why the extract emulsion should act more strongly is probably because it contains more protein adherent to the suspension of bacilli, but we are investigating this further, since individual variations in sensitiveness may play a part.

Old tuberculin: The old tuberculin Kochii was found quite inactive for toxic injections in animals sensitized to water extract of tubercle bacilli. It was attempted in eight sensitized animals in doses from .2 to .5 cubic centimeter, both in diluted and concentrated form. When injected post-orbitally undiluted there was some irritation caused doubtless by the glycerin but no typical symptoms of anaphylaxis. One sensitized guinea-pig died in thirty seconds possibly from this cause, after a dose of .3 cubic centimeter old tuberculin 153, followed immediately by unconsciousness and clonic convulsions. The reason why it should not act may not be entirely clear, yet the fact that heat to boiling has been used in its preparation offers one explanation Sufficient alteration of the specific protein is probably thus produced to prevent its action on animals sensitized with unheated extracts. Analogous conditions hold for the precipitin reaction in comparing old tuberculin and bacillary extract in testing precipitin sera (see Studies from Saranac Laboratory, 1900–4, p. 79).[32] When animals have been sensitized with old tuberculin they react with anaphylactic symptoms from the homologous tuberculin according to Marie and Tiffeneau[12] who worked with rabbits. The experiments of Lewis,[13] however, showed that guinea-pigs thus sensitized react equally well to evaporated broth. This fact naturally prevents any conclusion as to the specific relation these results have to bacillary products. Lewis regards this as evidence that the true tuberculin reaction involves a different factor than that which produces anaphylaxis. Our experiments with heated bacillary extracts have not been carried far enough to cover all these points, but a few guinea-pigs which have been sensitized with extract which was dried at 50° C. and then heated to 90° and 120° C., were not less anaphylactic than those sensitized with unheated extract.

This conforms to the experiences of others with serum proteins where coagulation is avoided by drying before heating. Some of our first experiments were done with heated extracts, but they were mixed with unheated portions.

Effect of bovine bacillary extracts on guinea-pigs sensitized with human bacillary extract. — The well-known specificity of the anaphylactic reaction has suggested its employment as a means of differentiating proteins. It is not, however, delicate enough as a reagent to justify very extensive use, for group reactions are to be expected as in agglutinins. Rosenau and Anderson have already shown this to be true in relation to serum proteins. Nevertheless, since some have professed to see a marked difference between the products of the human and bovine bacillus, one might predicate that the respective extracts would not act reciprocally in the anaphylactic reaction. That such is not the case may be seen from a few examples:

Experiment 288. — Guinea-pigs sensitized with 2.5 cubic centimeters W.E. 160, tested after thirty-nine days:

No. 46.	Injected post-orbitally	.75 cc.	E.E. 161	(human)	= + + + †	2½′
" 47.	"	"	"	"	E.E. 161	"	= + + + †	2′
" 49.	"	"	"	"	E E. 162	(bovine)	= + + + †	1′
" 50.	"	"	"	"	W.E. 162	"	= + + +	recovery.
" 51.	"	"	"	"	W.E. 162	"	= + + + †	1½′

Result: It is evident that the proteins of the human and bovine bacillus are analogous in the sensitizing and toxic substances necessary to produce anaphylaxis. That other acid fast bacilli are not so related is suggested by a few experiments detailed in the next paragraph.

Effect of human bacillary extracts on guinea-pigs sensitized with Timothy bacillus extract and vice versa. — In these experiments sixteen guinea-pigs were injected intraperitoneally with water extract 157 (Timothy) and eight with water extract 152 (human). Owing to an epidemic of enteritis only a few of the animals were available for toxic injections. Other animals of this series were tested, but on account of their poor condition are not included. Most of them failed to react and soon died or were killed.

Experiment 280, 16–VII–09:

No. 1. Sensitized B. Tim. Ext.; tested on 34th day, .75 cc. E.E. 157 (Tim.) post-orbitally = + + + † 5′

No. 6. Sensitized B. Tim. Ext.; tested on 34th day, .75 cc. E.E. 158 (human) = no symptoms.

No. 19. Sensitized B. Tim. Ext.; tested on 34th day, .75 cc. E.E. 157 (Tim.) = + + + recovery.

No. 10. T.B. Ext.; tested on 42d day, .65 cc. post-orbitally E.E. 158 = +

No. 11. " " " " " .5 " " E.E. 154X = + + + † 3′

No. 14. " " " " " .75 " " E.E. 157 (Tim.) = no symptoms.
after 15′ .5 cc. left eye E.E. 154X = + + + † 5′

No. 16. " " " " " .5 cc. E.E. 154 X = + + + recovery.

Result: In pig No. 14 it was demonstrated that the Timothy bacillary emulsion had no effect, while the homologous human bacillary emulsion caused fatal anaphylaxis within fifteen minutes. If the proteins were nearly related the animal would doubtless have been refractory to the second injection.

Further experiments on the reciprocal sensitizing and toxic effects of the avian and reptilian tubercle bacilli will be published later.

Relationship between the usual "tuberculin reactions" and anaphylaxis. — This is possibly one of the questions of first importance in the course of these investigations, and at the same time one of great difficulty to determine by experiments on the usual laboratory animals. While we have readily demonstrated the anaphylactic sensitiveness, the animals do not reveal the gross appearances of congestion seen in the tuberculin focal reactions in tuberculous tissues. The temperature variation is also not a safe criterion in guinea-pigs for judging a tuberculin reaction, being subject to fluctuations from slight causes. Both guinea-pigs and rabbits are relatively insusceptible to the various tuberculin tests, and respond only to comparatively large doses when tuberculosis is present.

In healthy — non-tuberculous — rabbits and guinea-pigs repeated injections of old tuberculin or of filtered extracts

of tubercle bacilli have not usually been observed to produce fever. Possibly a more frequent observation of the temperature and daily injections might reveal a febrile change such as found by Vaughan, Wheeler and Gidley[14] with egg white and typhoid poison. Only one instance of febrile disturbance in non-tuberculous animals produced by tuberculin is known to me, and that is in connection with healthy cattle injected at intervals of six to ten days.

Calmette[15] observed that three successive intravenous injections of .50 gram of his filtered precipitated tuberculin ("T.L.") caused fever in healthy bovines (non-tuberculous?). It should be noted also that this tuberculin contains broth proteins, especially the peptone or albumoses. It is also true that von Behring found his immunized calves free from tuberculous foci, yet they reacted to tuberculin for many months after the inoculation of bovo-vaccine, which consists of living bacilli of low virulence. Both these observations are open to the objection that it is almost impossible to exclude the presence of concealed tubercles and it is well known that the smallest foci may cause a tuberculin reaction. Moreover, the first injection often fails to excite a fever response, whereas repetition of the same dose may produce one when tubercles are present. Certain stages of lymphatic tuberculous infection when no tubercles are detected are to be classed with actual tuberculosis if they react to tuberculin, because in all probability the glands harbor tubercle bacilli. In any case, the simple lymphatic hyperplasias regarded by von Behring and Bartel as the scrofulous changes predisposing to later infection, cannot be exactly compared with sensitiveness acquired by a single injection of filtered tuberculo-protein.

It was therefore of especial interest to determine the effects of tuberculin applied as in the usual diagnostic methods to these animals in which it was certain that tubercles were absent. As already noted (p. 38), the temperature varied in guinea-pigs after the toxic injections; sometimes there was elevation but more often depression. The injections were always large enough to kill tuberculous guinea-pigs, yet in the non-tuberculous which survived the

anaphylactic symptoms, only a few died later from collapse and the rest showed only temporary depression.

Since there were no focal reactions with their accompanying grave extravasations in the viscera, there is reason to think that this fact may be sufficient to account for the slight secondary effects on the non-tuberculous animals. To state the matter differently, the mechanism might be identical, but mechanical or circulatory changes might account for the different results. Nevertheless these comparisons do not lead to any conclusion about the real differences, if any, between them. It was therefore necessary to compare the local tuberculin tests for further information. Unfortunately, neither the cutaneous nor the conjunctival reactions in guinea-pigs and rabbits give decisive results in tuberculous animals and cannot be depended upon for this comparison.

Fortunately, the intradermic test introduced by Mendel [16] and Moussu and Mantoux [17] has been adapted to guinea-pigs and cattle with greater success by Roemer,[18] who prefers the term "intracutaneous" to designate the method. According to Roemer it is of great diagnostic value in these animals. The technic which he describes was used in our experiments with guinea-pigs.

Intradermic tuberculin test. — A twenty per cent solution in N/NaCl of O.T. 153 was prepared and for control a twenty per cent solution of evaporated broth of the same concentration as the O.T. The dose injected was .1 cubic centimeter of each (= .020 cubic centimeter O.T.) simultaneously. Besides these solutions the same water extract used for sensitizing was used in the same way. Opposite sides of the animal were shaved clean over an area of 2.5 to 4 cubic centimeters, selecting where possible places where the hair was white or at least light hued. The control broth was injected on the left and the tuberculin on the right side. A reaction was recorded only when a distinctly greater area of induration and redness could be detected when compared with the control spot (Table XV.).

The temperature was not observed.

TABLE XV. (a). (Experiment 291, 8-XII-09.)

Guinea-pigs inoculated subcutaneously with .1 cubic centimeter very weak emulsion II 37. Mild infection with slow course.

No.	Weight.	Interval.	O.T. 153, 20 Per Cent.	Reaction: Induration and Redness.		Control Broth, 20 Per Cent.	Reaction: Induration and Redness.	
				24 Hours.	48 Hours.		24 Hours.	48 Hours.
1	410	27 days.	.1 cc.	+	+<	.1 cc.	+	Trace.
3	345	27 "	.1 "	+	++	. . . . .	+	"
4	275	27 "	.1 "	+	+<	. . . . .	+	"
5	365	27 "	.1 "	++	+++	. . . . .	+	O
12	400	40 "	.1 "	++	+++	. . . . .	Trace.	O
13	380	40 "	.1 "	+	+++	. . . . .	"	O
15	290	40 "	.1 "	O	++	. . . . .	"	O

TABLE XV. (b). (Experiment 288, 16–IX–09.)

Sensitized by 2.5 cubic centimeters T.R. emulsion 158 intraperitoneally.

No.	Weight.	Interval.	O.T. 153, 20 Per Cent.	Reaction: Induration and Redness.		Control Broth, 20 Per Cent.	Reaction: Induration and Redness.		Post-orbital Test for Anaphylaxis.	Result.
				24 Hours.	48 Hours.		24 Hours.	48 Hours.		
35.	300	48 days.	.1 cc.	O	O					
36.	365	48 "	.1 "	O	O					
37.	280	48 "								
38.	370	48 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 cc. E.E. 161	+++† 2½'
39.	270	48 "	.1 cc.	O	O	. . . . .	. . . . .	. . . . .	.75 " " "	+++†3'
40.	290	62 "	.1 "	+	+ (10 mm.)	.1 cc.	Trace.	O		
41.	310	48 "								
42.	(Pregnant.) 410	62 "	.1 "	+	+	.1 "	"	Trace.	.75 " " "	++; killed.

TABLE XV. (c). (Experiment 288, 6-X-09.)

Sensitized with 2.5 cubic centimeters W.E. 159 intraperitoneally.

No.	Weight.	Interval.	O.T. 153, 20 Per Cent.	Reaction: Induration and Redness.		Control Broth. 20 Per Cent.	Reaction: Induration and Redness.		Post-orbital Test for Anaphylaxis.		Result.
				24 Hours.	48 Hours.		24 Hours.	48 Hours.			
21 . .	410	81 days.	.1 cc.	++9 mm.	+> 5 mm.	.1 cc.	Trace.	0	86 days.	.75 cc. E.E. 161	No symptoms.
23 . .	430	81 "	.1 "	+5 mm.	+> 5 mm.	.1 "	"	0	86 "	.75 " " "	+++† 3′
25 . .	240	81 "	.1 "	+7 mm.	0 3 mm.	.1 "	"	0	86 "	.75 " " "	+++† 2′
	29-XI-09										
61 . .	300	49 "	.1 "	Trace.	0	.1 "	"	0			
62 . .	300	49 "	.1 "	"	0	.1 "	"	0			
63 . .	280	49 "	.1 "	"	0	.1 "	"	0			
	(Pregnant.)										
64 . .	400	49 "	.1 "	"	0	.1 "	"	0			
65 . .	300	49 "	.1 "	"	0	.1 "	"	0			
18 . .	400	79 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++† 2′
19 . .	290	79 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++† 1½′
22 . .	320	76 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++ killed.
28 . .	450	86 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++† 3′
29 . .	420	86 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++† 2′
57 . .	510	42 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	No symptoms.
58 . .	350	42 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+++† 2′
59 . .	420	42 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+?
60 . .	375	42 "	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	. . . . .	.75 " " "	+

Result: The tuberculous guinea-pigs all reacted slightly twenty-seven days after infection, but stronger after forty days. The induration and areola of redness was too diffuse to admit of accurate measurement in all cases so the reaction was recorded by signs. The papules varied from seven to twenty millimeters in diameter, but the control broth produced irritation enough to cause an areola of redness five to ten millimeters in diameter. It is significant that the latter had generally subsided after forty-eight hours, while the tuberculin papules persisted or became larger.

Very much less reaction was produced in the sensitized animals, yet two of the T.R. pigs after sixty-two days and three of the extract pigs after eighty-one days responded slightly. Those tested after forty-eight to forty-nine days were all negative. Even those which appeared to react showed less after forty-eight hours, quite contrary to the behavior of the tuberculous animals. To show the existence of anaphylactic sensitiveness in the non-tuberculous pigs, a number were tested near the same intervals. It would be premature to conclude from so few animals that sensitization of the skin to ordinary tuberculin is not produced by the water extracts peritoneally injected.

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Enough is shown in these experiments to indicate a difference between the infected animals and those simply anaphylactic, in relation to old tuberculin applied in this way. We shall publish further observations in which the bacillary extract is used instead of old tuberculin. Some preliminary tests on normal guinea-pigs produced so much irritation that it is necessary to find out the minimum amount of our preparation required to react tuberculous animals without affecting normal animals.

The question whether healthy or non-tuberculous individuals can be sensitized by tuberculin so as to react to subsequent injections is a practical one. In connection with the local or integumental tests there is a difference of opinion based on the results of repetition of the conjunctival or cutaneous tests on the same eye or skin area. A marked increase in local susceptibility is at least developed when

tuberculin is applied at the same place. Whether the individual has or has not had tuberculosis is a moot question in these cases. Certainly the latent susceptibility in some infected individuals gives little or no response to the first test, but may be roused to severe reaction at the second. On the other hand, the well-known cutaneous manifestations of serum disease and hypersensitiveness to other proteins gives by analogy support to the belief in the possibility of sensitizing the skin by tuberculin when applied locally to non-infected individuals. So far as our experiments go they confirm this belief and further indicate that a general skin sensitization may be produced by intraperitoneal injections, though less marked than that present in tuberculous animals. It can easily be argued, too, that since anaphylactic sensitiveness can be established in non-infected animals to the tuberculo-protein, there is every reason to expect some cutaneous manifestation of hypersusceptibility similar to that which other proteins produce in animals sensitized with the homologous protein.

As already noted, general or febrile reactions do not follow the repetition of injections of ordinary diagnostic doses of tuberculin into non-infected individuals and thus lead to errors in diagnosis; yet it is conceivable that some specific sensitiveness ("allergy") may be conferred in this way. Practically, it has not been discovered, but were the amounts and intervals known when the maximum effect was present it might be recognized by an intradermic test. Moreover, it cannot be said that the subcutaneous injection of tuberculin into cattle when repeated has led to febrile reactions and a consequent diagnosis of tuberculosis which did not exist. Many herds have yearly tests with negative results.

Nevertheless, these observations show the care needful in interpreting positive reactions after repeated tuberculin injections.

Part II.

Transferred susceptibility to tuberculo-protein. — Shortly after the publication by Otto [19] and Gay and Southard [20] of their discovery of the passive transfer of serum sensitiveness from one animal to another, the writer began some experiments in tuberculosis, hoping to find a new method of diagnosis by the use of this principle. These attempts were made (October, 1907) before a method was found to demonstrate tuberculo-anaphylaxis satisfactorily and thus came to nothing. Serum collected from healthy and tuberculous persons was injected subcutaneously into fourteen young guinea-pigs in doses of .2 to .2 cubic centimeter. After three days they received intraperitoneally 1.5 cubic centimeters to two cubic centimeters of concentrated water extract of tubercle bacilli 148 B and C without showing characteristic sensitiveness.

Homologous blood: guinea-pig to guinea-pig. — The transfer of fresh blood from tuberculous guinea-pigs to young healthy guinea-pigs was also tried without definite result in twelve animals. Large quantities (from one to ten cubic centimeters) were injected intraperitoneally and in some instances intracardially, mixed with an equal bulk of one per cent sodium citrate solution. Two, three, and sixteen days later 1.25 cubic centimeters water extract 148D was given intraperitoneally. Two died in six to eight hours of collapse which was suggestive of susceptibility, but one out of four controls also succumbed. U. Friedmann [21] also failed in similar experiments. In some transferred serum experiments two out of seven animals similarly injected died in twelve hours, but the others showed no symptoms of anaphylaxis. Some animals were injected intracardially twenty-four and again forty-eight hours after blood transfer, but with indifferent result. Generally the blood and serum of tuberculous guinea-pigs appeared free from any reacting

substance that could be transferred. Cutaneous tuberculin tests, however, were not made in this experiment.

Organ cells. — The thought that the reacting substances might be contained in the cells led us to try injection of emulsions of the tuberculous organs and also of the brain of tuberculous guinea-pigs followed by tests for anaphylaxis. Some animals were given emulsions of liver, spleen and lymph nodes containing tubercles. Others received leucocytes and brain emulsion. The latter appeared to cause some slight susceptibility in two pigs out of four injected post-orbitally three days later. The others were negative, but only a few experiments were done along this line, so that no great stress can be laid on the results.

Rabbit to rabbit. — Quite analogous experiments were made with the blood of tuberculous rabbits transferred to others, with the object of passively transferring the fever-reacting power. No success was attained in a few trials where the animals were injected intravenously with the blood, and forty-eight hours later with W.E. 148D.

Rabbit to guinea-pig. — Four guinea-pigs injected intraperitoneally with five and eight cubic centimeters of blood of tuberculous rabbits all died in twenty-four hours without being tested with tuberculin. They were evidently strongly irritated by the large amount of alien blood. The experiment was repeated with three guinea-pigs which were tested three, four, and six weeks later post-orbitally without symptoms of anaphylaxis.

Human to guinea-pigs. — The first attempts to transfer human serum to guinea-pigs and thereby confer sensitiveness had failed when tested by the former methods in use. After we adopted the post-orbital route for the toxic injection the attempt was renewed. By the use of larger amounts of blood taken from the veins of tuberculous patients it was

hoped to passively transfer a specific sensitiveness. The blood was drawn into one per cent sodium citrate and promptly injected into guinea-pigs in amounts of three to eight cubic centimeters of the mixture of which five-eighths was pure blood. A few received serum only and some were injected post-orbitally.

After an interval varying from twenty-four hours to ninety-seven days they were tested post-orbitally. A total of twenty-four guinea-pigs was injected with blood of sixteen patients, chiefly from the advanced class. Only a few animals evinced symptoms of anaphylaxis as shown in the table.

TABLE XVI. (Experiment 260D.)

No.	Weight.	Case.	Stage.	Blood, Intraperitoneally.	Interval, Days.	Test, post-orbital.	Result, Symptoms Anaphylaxis.	Death.	Autopsy.
34.	25-I-09. 285	S.—	Prog. II.	3 cc.	14	.5 cc. E.E. 152.	O	Killed 3-III.	No tuberculosis; perisplenitis.
35.	360	"	" "	8 "	4	.5 " " "	O	" "	" " normal.
36.	365	T.—	Control, healthy.	3 "	(Notes omitted.)			" (?).	" " "
37.	425	"	Control, healthy.	9.5 "	4	.5 cc. E.E. 152.	O	" "	" " perisplenitis.
39.	26-I-09. 480	B.—	Prog. II.; acute.	3 "	13	.5 " " "	+(?) Defecation and urination.	Died in night.	" " few areas collapsed lung.
40.	390	"	Prog. II.; acute.	1.5 "	24	.5 " " "	O	Killed 19-II.	No tuberculosis; normal.
41.	500	"	Prog. II.; acute.	8.25 "	13	.5 " " "	+ ? Cough; deep respiration.	" 3-III.	" " perisplenitis.
43.	6-II-09. 380	L.—	Prog. III.	3 "	20	.5 " " "	O	" 8-III.	" " peritoneal hypertrophy.
44.	430	Ca.—	Prog. II.	3 "	20	.5 " " "	O	"	No tuberculosis; perisplenitis.
45.	400	O'C.—	Chron. III.	3 "	20	.5 " " "	+ ? Cough; deep respiration.	"	" " "
46.	I-III-09. 460	H.—	II. Arrested.	5 "	36	.5 " " "	O	" 6-IV.	" " normal, pregnant.
47.	575	"	II. Arrested.	Post-orbitally. 1 cc.	36	.5 " " "	+ ? Cough; deep respiration.	"	No tuberculosis; slight epicardial hemorrhage?

TABLE XVI. (Experiment 260D.) — *Continued.*

No.	Weight.	Case.	Stage.	Blood, Intraperitoneally.	Interval, Days.	Test, post-orbital.	Result, Symptoms Anaphylaxis.	Death.	Autopsy.
48.	710	A.—	II. Arrested.	Intraperitoneally. 5 cc.	15	.5 cc. E.E. 152.	O	Killed 14-IV.	No tuberculosis; normal.
49.	400	"	II. Arrested.	Post-orbitally. .75 cc.	15	.5 " " "	O	" "	" " "
50.	240	W.—	Chron. II.	Intraperitoneally. 5 cc.	O	O	—	Died; collapse in night.	Blood unabsorbed in peritoneal cavity.
52.	9-III-09 550	K.—	Prog. II.	5 "	32	.5 cc. E.E. 152.	O	† in night.	No tuberculosis; beginning pregnant (3).
53.	730	R.—	Prog. III.	5 "	32	.5 " " "	+ (?) Cough; slight respiratory disturbance.	Killed 10-IV.	No tuberculosis; normal.
54.	220	O'C.—	Chron. III.	Post-orbitally. .5 (serum)	2	.5 " " "	O	" 11-III.	" " "
55.	215	"	Chron. III.	" " Intraperitoneally. 1.5 cc.	32	.5 " " "	O	" 27-IV.	" " "
56.	485	"	Chron. III.	Post-orbitally. .75 cc. (serum)	32	.5 " " "	Cough. O	" 12-V.	" " "
57.	205	M.—	III. Mening. Moribund.	Intraperitoneally. 4 cc.	40	.5 " " "	+ (?) deep breathing.	" 28-IV.	Small caseous nodules on omentum; tuberculosis +.
58.	. . . . .	Ca.—	Chron. III.	5 "	47	.5 " " "	O	" 12-V.	No tuberculosis; focal necroses liver.
59.	. . . . .	A.—	Quiesc. II.	5 "	47	.5 " " "	O	" 12-V.	No tuberculosis; normal.
62.	. . . . .	W.—	Quiesc. I.	5 "	97	.75 " " 154X.	O	" 30-VIII.	" " peritoneal adhesion.

Result: Four animals gave suggestion of anaphylactic symptoms, but they were not pronounced enough to warrant the name. Two died after the toxic injection, but whether because of sensitization or not is uncertain.

The net result of these experiments gives very slight evidence of passively transferred anaphylaxis to the guinea-pig from tuberculous human blood.

Human to young rabbits. — Similar experiments on young rabbits have appeared successful, as shown by Yamanouchi,[22] who also introduced this as a means of diagnosis.

The blood injected intraperitoneally, followed in twenty-four hours by the toxic injection of old tuberculin intravenously, caused convulsions and death in a few seconds in many rabbits weighing between five hundred and seven hundred grams. Frequently these symptoms appeared first at the second injection of tuberculin after forty-eight hours. Control animals injected with blood from patients suffering from other diseases had no anaphylactic symptoms on injection with tuberculin. Larger doses of tuberculin alone were, however, capable of producing death in a similar way according to Yamanouchi, which fact makes the value of this method in diagnosis open to question. A further mention of this point will be given in the contribution by Dr. Krause.

In a few cases the diagnostic value of this test was apparently confirmed in our hands. Some of the same blood used in the experiments in Table XVI. was also injected into young rabbits which were later given pure water extract of tubercle bacilli or the emulsion unfiltered, as indicated below. Sodium chloride was always present in .85 per cent. Tuberculous guinea-pig and rabbit blood was tried in two animals. Crude tuberculin was not used because of its content of extraneous glycerin, peptone, and meat extractives. Yamanouchi also admits that his best results were obtained from an extract of powdered tubercle bacilli

residue remaining after the preparation of old tuberculin. This was centrifugalized but not filtered; hence his preparation was similar to Koch's T.R. and contained little dissolved protein. Our extract emulsion resembled of course the T.O. of Koch and consequently was more active than the bacillus extract described by Yamanouchi. It seemed to us inadmissible to form conclusions from injections of old tuberculin or any broth extract of tubercle bacilli even in the small doses .5 to one cubic centimeter of O.T., diluted to five cubic centimeters of N/NaCl.

TABLE XVII. (Experiment 260C.)

No.	Weight.	Case.	Disease Stage.	Blood, Intraperitoneally.	First Toxic Injection, 24 Hours Intravenously.	Result.	Second Toxic Injection, 48 Hours Intravenously.	Result.	Autopsy.
Belgian hare.	15-XII-08.								
1	545	F.R.—	Healthy.	5 cc. + 2 cc. Na Citrate 1%.	2 cc. W.E. 151A.	O	5 cc. W.E. 151A.	O	Killed; normal.
3	420	B.—	Quiescent tuberculosis II.	4 cc. + 2 cc. Na Citrate 1%.	2 " " "	O	5 cc. W.E. 151A.	†2' convulsions.	Normal; heart distended.
6	670	C.—	Chronic tuberculosis II.	7 cc. + 3 cc. Na Citrate 1%.	2 " " "	O	5 cc. W.E. 151A.	O	Killed; normal.
4	390	Control.	. . . . .	O	2 " " "	O	5 cc. W.E. 151A.	O	" "
5	800	B.—	Latent tuberculosis I.	3 cc. + 2 cc. Na Citrate 1%.	5 " " "	†2' convulsions.	. . . . .	. . . . .	Some blood remains in peritoneal cavity; normal.
2	855	Control.	. . . . .	O	10 cc. E.E. 152.	†1'	. . . . .	. . . . .	Some coccidiosis in liver; otherwise normal.
9	12-I-09. 565	L.B.—	Healed tuberculosis I.	4 cc. + 3 cc. Na Citrate 1%.	5 " " "	O	5 cc. E.E. 152.	O	Killed later; normal.
0	585	D.S.—	Healed I.	5 cc. + 3 cc. Na Citrate 1%.	5 " " "	O	5 " " "	O	" " "
1	585	A.S.—	Chronic tuberculosis II.	5 cc. + 3 cc. Na Citrate 1%.	5 " " "	†2' convulsions.	. . . . .	. . . . .	Normal.

TABLE XVII. (Experiment 260C.) — *Continued.*

No.	Weight.	Case.	Disease Stage.	Blood, Intraperitoneally.	First Toxic Injection, 24 Hours Intravenously.	Result.	Second Toxic Injection, 48 Hours Intravenously.	Result.	Autopsy.
Rabbit.									
8. . . .	420	Tuberculous guinea-pig.	III.	5 cc. + 5 cc. Na Citrate 1%.	3 cc. E.E. 152.	O	5 cc. E.E. 152.	O	Killed later; few caseous spots in mesentery.
Hare.									
42. . . .	1340	Tuberculous hare.	Miliary III.	11 cc. (serum)	10 " " "	† 4′	. . . . .	. . . . .	Coccidiosis in liver.
51. . . .	745	W.—	. . . . .	5 " "	5 " " "	† 5′	. . . . .	. . . . .	Some extravasation right costal pleura; coccidiosis in liver.

Result: Two cases of latent tuberculosis and two of a chronic type appeared to sensitize rabbits; one chronic and two healed cases failed. One control succumbed to a large dose of the emulsion; two controls failed as did the guinea-pig blood. The rabbit was positive, but to a large toxic injection.

Whether the positive results can be regarded as proof of transferred specific sensitiveness or not certainly depends on individual factors in the animals to some extent, for we found later that small amounts (one cubic centimeter to two cubic centimeters) of the extract emulsion were able to produce similar symptoms and death in some animals (see Krause). So far as we could observe, the symptoms were quite similar to sudden shock. A few gasps, dilatation of the pupils, sudden unconsciousness and convulsive movements ushered the end. The heart continued to beat faintly after complete respiratory failure and relaxation. No extensive respiratory movements were usually noted, nor were extravasations found in the cardiac walls or diaphragm as in anaphylactic guinea-pigs. It is also important that the intravenous injections be made with warmed solutions which are isotonic to the blood. Altogether the matter involves considerable difficulty in interpreting results, and doubtless the use of several animals for each specimen of blood injected for diagnosis in order to insure correct conclusions.

Eitner and Stoerk[23] were unable to confirm the results of Yamanouchi. Careffa[24] claims to have succeeded. More recently Roepke and Busch[25] published their results with sixteen cases including mostly pulmonary tuberculosis stages II. and III. with completely negative results. They used both Hœchst old tuberculin and tuberculin residue extract, and by control injections suggest that traces of phenol might have caused positive results since they produced the symptoms described by Yamanouchi when phenol .5 per cent was added to the tuberculin. This explanation has no weight in our opinion, because one can produce the convulsions and death with the pure extract emulsions in normal saline. On the other hand, we should not expect the transfer of a

reacting substance from the blood of advanced, progressively tuberculous individuals with any such uniformity as was obtained by Yamanouchi, if our results on tuberculous guinea-pigs form any guide.

It is probable that heterologous blood does not readily sensitize by passive transfer, judging from the experiments of Novotný and Schick.[26] They found that the serum of children who were sensitive to horse serum did not transfer this to guinea-pigs. Other experiments seem to have shown that the fever susceptibility and cutaneous reactivity to tuberculin may be transferred. Lesné and Dreyfus[27] have also tried to prove the passive transfer to guinea-pigs by intracerebral injections of tuberculin. They succeeded in obtaining evidence of an increased sensitiveness in twenty per cent of the tests, but did not think it of diagnostic value. They failed to transfer fever reactivity from tuberculous individuals to guinea-pigs and rabbits. Bauer[28] claims to have succeeded, but Novotný[29] showed the fallacy of this by obtaining the same temperature elevations after non-tuberculous sera plus tuberculin injections.

Helmholz[30] succeeded in transferring cutaneous reactivity using von Pirquet's test as a criterion. Attention has been drawn to the uncertainty of these methods in Part I., and there is on the whole little evidence of a passively transferred tuberculin reactivity as distinguished from acute anaphylaxis.

Review and conclusions. — It will be worth while to review our work thus far and consider whether it has assisted in the clearer understanding of the pathology of tuberculosis and the means which nature uses to combat the disease. It seems to us that it does; and it emphasizes still more the wide range of diseases and symptoms to which the phenomena of protein sensitization apply.

There can be no question that much of the symptomatology and pathological changes in the tuberculous rests on the local and general sensitization of the infected individual. One must forbear to assume that the protein substance of

the tubercle bacillus is alone responsible for the fever, coagulation necrosis or other results of the disease; yet this study gives us an insight as to its importance.

It is especially important that non-tuberculous animals as well as tuberculous undergo a sensitization which probably persists a long time, and is transmitted to offspring by the mother. To be sure the evidence of this sensitization has not been so delicate as in the tuberculous animal which, in addition to the anaphylaxis in the narrow meaning, has marked focal and febrile reacting power. Yet one must reflect that the method of sensitization is so unnatural and crude by injection of such large amounts of bacillary products at one time that we can only claim that one form of sensitization is possible by this method.

From what we know of natural infection it would appear that a very small amount of bacillary substance was sufficient to sensitize an animal enough to react to the tuberculin tests, and coincidentally to the acute anaphylactic symptom-complex. The latter is of course only a presumption based on our experiments with artificially infected animals. We have not sought to determine the least amount of the tuberculo-protein capable of sensitizing guinea-pigs nor the method of producing the greatest sensitiveness.

From our results in Table II. it can be seen that one dose of .1 cubic centimeter containing less than .0008 gram dry protein was the least amount that we actually found sufficient. As compared with beef serum, which for the same bulk we estimated to be five times heavier in dry protein, it apparently requires more tuberculo-protein to sensitize to a perceptible degree. It is here to be remembered that tuberculo-precipitin formation is feeble as compared with serum antigen; and a weaker chemical affinity may play the chief part in the difference in sensitization.

On the other hand, we have not imitated the conditions of absorption from foci of disease in our experiments and cannot be sure that the very small amounts of protein dissolved out of the bacillus, and gradually absorbed, would not represent far less in the aggregate than the smallest single

dose found to be effective. Arthus[31] indeed found that the same amount of serum in divided doses was more active in sensitizing.

It was shown that our tuberculous guinea-pigs as a rule had less sensitiveness to the anaphylactic shock than to the usual tuberculin reaction. On the theory of exhaustion of antibodies this could be accounted for, but it would be of interest to try gradual sensitization without formation of tubercles and the resulting changes due to disease. In former experiments[32] we had failed to sensitize hares to a noticeable degree by enclosing bacilli in sealed capsules of porous filter clay which were introduced into the peritoneal cavity. Neither temperature nor local reactions about the encysted capsules were definitely observed when the capsules were intact. More recently, however, Moussu[33] has accomplished it by the same method applied to larger animals,—dogs, sheep, goats, and especially cattle. Heymans[34] also claims that the immunity in cattle produced by his method of implantation of reed capsules under the skin is accompanied by a tuberculin fever sensitization which persists until the capsule is no longer capable of diffusing specific substances. It does not appear whether or not we have to do with a different kind of sensitization in such experiments than the anaphylaxis of the acute type found in our present work. Presumably, the diffused substances are chiefly protein in character, and until further study we cannot assume any fundamental difference in its effect from our extracts. When we consider our results upon the progeny of tuberculous and sensitized females respectively there is an argument for a difference, because of the absence of any appreciable sensitiveness in the young of the former and the very great susceptibility of the latter. Clinical experience on the newly born from tuberculous mothers also indicates a lack of cutaneous and fever sensitiveness transmitted to the child, which is hard to understand if acute anaphylaxis and tuberculin hypersusceptibility have a common origin and mode of development. However this may be, the important

fact has been developed that a specific property is transmissible to the young while hitherto no satisfactory proof has been furnished of any kind of specific inheritance in relation to tuberculosis. It is true that Carrière[35] has found the young of parent guinea-pigs which were treated with distillates of cultures and with ethereal, toluol and xylol bacillary extracts more sensitive to inoculation than controls, but the published details do not appear sufficiently convincing. The same objection also applies to the experiments of Sciolla and Palmieri,[36] which purported to show an increased sensitiveness to fatal doses of tuberculin in the young of tuberculin injected guinea-pigs. We have not progressed further in this field and cannot therefore assume anything about the resistance of the sensitized offspring to infection.

The influence of anaphylactic sensitiveness on the course of infection is now being investigated in this laboratory.

The question of passively transferred sensitiveness seems to us still open so far as the specificity of the reactions is concerned, which are used to demonstrate it in tuberculosis. There should be some way worked out, and probably will be, by which it can be clearly shown and thereby have a future use in diagnosis.

No attempt will be made at present to discuss the theories of anaphylaxis, as this work has not aimed to explain anaphylaxis but to discover what special relations the phenomena have to tuberculosis. Inferences may be drawn, however, that so far as the foregoing experiments bear on the mechanism, the symptoms could be accounted for by changes in the cells without the presence of any free antibody or ferment in the blood. It is of course probable that some element is present in the blood, and future experiments will reveal it by unmistakable evidence of transferred anaphylaxis. It would seem to be far weaker in quality or amount than the corresponding factor in transferred serum anaphylaxis.

The chief points in this work may be gathered together as follows:

CONCLUSIONS.

1. A true anaphylactic sensitization can be produced in guinea-pigs by the pure protein substance of the tubercle bacillus extracted by water at 50° C.

2. Intradural toxic injections through the optic foramen produce all the symptoms of fatal anaphylactic shock with respiratory stimulation and paralysis. Intravascular toxic injections are also effective but less typical symptoms follow, while intraperitoneal toxic injections are practically negative in result.

3. A single injection of tuberculo-protein equal to .0008 gram dry weight is sufficient to sensitize a normal guinea-pig; probably even less is required.

4. Tuberculous animals are sensitive but not uniformly so to acute anaphylaxis tests. There is no absolute relation between the degree of sensitiveness and stage of the disease. As a rule it is lost in the far advanced stage. Repeated inoculation increases it.

5. Acute anaphylaxis could not be demonstrated in the offspring of inoculated guinea-pigs, but is very uniformly produced in those of sensitized mothers injected during pregnancy. This transmitted sensitiveness is very marked. It is not demonstrated whether it is actively produced in the fetus or passively conveyed through the placental blood.

6. Sensitized guinea-pigs which survive a toxic injection become refractory to a subsequent one for a time, or at least react less strongly. The temperature is not regularly elevated or depressed after toxic injections.

7. Sensitization is possible with both the filtered bacillary extract and the well-washed residue "T.R."

8. Toxic injections are more effective with an unfiltered, centrifugalized extract (= "T.O.") than with the filtrate; old tuberculin was uncertain.

9. Bovine bacillary extract is very toxic to guinea-pigs sensitized with human bacillary extract, but the latter was not toxic to animals sensitized with B. Timotheus extract; that is, the human and bovine proteins act reciprocally, but the Timothy bacillus protein does not.

10. Sensitized normal guinea-pigs do not react to ordinary tuberculin like tuberculous guinea-pigs, yet some respond slightly to an intradermic test. This difference between anaphylactic sensitization and tuberculin reactivity need not be fundamental, as it is possibly due to another factor as yet undetermined.

11. Transferred or passive anaphylaxis was unsuccessful from tuberculous guinea-pigs to sound ones; also from rabbit to rabbit and from rabbit to guinea-pig. From human to guinea-pig the results were uncertain but to rabbit partially successful.

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REFERENCES.

1. Baldwin, E. R., and Price, J. W. Cent. f. Bakt. (Ref.), 1906, xxxviii, 751.
2. Bulletin No. 36, Hyg. Lab. U.S.P.H. and M.H.S., 1907.
3. Vaughan, V. C. Boston Med. and Surg. Journ., 1906; Journ. of Infect. Dis., 1907, iv, 476.
Vaughan, V. C., Jr. Journ. Amer. Med. Assoc., 1905, 1340.
4. Otto, R. Handbuch d. Pathog. Mikroörg., Kolle and Wasserman, 1908, ii, 255.
5. Rosenau and Anderson. Journ. of Infect. Diseases, 1908, v, 85.
6. Anderson and Rosenau. Arch. of Intern. Med., 1909, iii, 519.
7. Baldwin, E. R. Yale Med. Journ., 1909, xv, 257.
8. Gay, F. P., and Adler, H. M. Journ. Med. Research, 1908, xviii, 433.
9. Gay and Southard. Journ. Med. Research, 1907, xvi, 143.
10. Besredka, A., and Steinhardt, E. Annáles d. l'Inst. Pasteur., 1907, xxi, 384.
11. von Lingelsheim. Deutsch. med. Woch., 1898, 583.
12. Marie, A., and Tiffeneau, M. C. r. d. l. Soc. d. Biol., 1908, lxiv, 501.
13. Lewis, P. A. Arch. of Clin. Med., 1909, iv, 528.
14. Vaughan, V. C., Wheeler, L. M., and Gidley, W. F. Journ. Am Med. Assoc., 1909, liii, 628.
15. Calmette, A. Sixth Internat. Cong. on Tuberc., 1908, i, Pt. 1, 219.
16. Mendel, F. Med. Klin., 1908, iv, 402.
17. Moussu, G., and Mantoux, C. C. r. Acad. d. Sci., 1908, cxli, 502.
18. Roemer, P. Beiträge z. Klinik d. Tuberk., 1909, xii, 185; xiv, 1.
19. Otto, R. Münch. med. Woch., 1907, 1665.
20. Gay and Southard. Journ. Med. Research, 1907, xvi, 143.
21. Friedmann, U. Münch. med. Woch., 1907, No. 49.

22. Yamanouchi, T. Wien. klin. Woch., 1908, No. 47, 1623.
23. Eitner and Stoerk. Wien. klin. Woch., 1909, No. 23, 810.
24. Careffa, V. Riforma Med., 1909, No. 45.
25. Roepke and Busch. Beiträge z. Klinik d. Tub., 1909, xiv, No. 2, 147.
26. Novotný, J., and Schick. Zeitsch. f. Immunitätsforsch, 1909, iii, 671.
27. Lesné, E., and Dreyfus, L. C. r. d. l. Soc. d. Biol., 1909, lxvi, 10.
28. Bauer, J. Münch. med. Woch., 1909, 1219.
29. Novotný, J. Zeitsch. f. Immunitätsforsch, 1909, iii, 679.
30. Helmholz, H. F. Zeitsch. f. Immunitätsforsch, 1909, iii, 370.
31. Arthus, M. Archiv. intern. de Physiol., 1909, vii, 471.
32. Trudeau, E. L., Baldwin, E. R., and Kinghorn, H. M. Journ. Med. Research, 1904, xii, 169.
33. Moussu, G. M. C. r. d. l. Soc. d. Biol., 1905, lix, 463; 1906, lxi, 95.
34. Heymans, J. F. Sixth Intern. Cong. on Tub., 1908, iv, 997.
35. Carrière, G. Arch. d. Med. Exper., 1900, xii, 782.
36. Sciolla and Palmieri. Semaine Med., 1896, 443.

(*b.*) STUDIES IN PASSIVE OR TRANSFERRED ANAPHYLAXIS.*

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SYNOPSIS.

I. INTRODUCTION.
   - A. — Transferred anaphylaxis as a clinical diagnostic measure (method of Yamanouchi).
   - B. — Attempt to apply it with serum of immunized cow.
   - C. — Technic.

II. A CONSIDERATION OF THE SYMPTOMS AND CRITERIA OF ANAPHYLAXIS AND RELATED CONDITIONS (HYPERSUSCEPTIBILITY).
   - A. — Acute symptoms.
   - B. — Gradual symptoms.

III. RESULTS OF EXPERIMENTS.
   - A. — With rabbits treated with serum of immunized cow.
   - B. — With rabbits treated with serum of normal cows.
   - C. — With untreated rabbits.
   - D. — With rabbits with acquired anaphylaxis.

IV. A DISCUSSION OF THE TOXIC EFFECTS OF VARIOUS PREPARATIONS OF BACILLARY PRODUCTS ON YOUNG RABBITS.

V. CONCLUSIONS.

The results of Yamanouchi's [1] experiments of sensitizing healthy young rabbits to tuberculin by previous treatment with the blood and serum of tuberculous human beings encouraged similar researches along related lines. In view of his work, there was à priori reason to expect that the serum of other animals that had undergone immunization to the bacillus tuberculosis or active treatment with the products of this organism (water extracts, old tuberculin, etc.), might possibly confer the same sensitiveness upon rabbits as would the serum of an individual who was afflicted with the disease.

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At the Saranac Laboratory for the Study of Tuberculosis several animals were available for a study of this kind. One cow (Cow No. 5) had been immunized against the human tubercle bacillus and for six or seven years had been periodically treated with tubercle bacillary extracts and emulsions. In December, 1902, and March, 1903, this cow received intravenous injections of a living "R1" culture, — 1.5 milligrams in the first instance and twenty-five milligrams in the second. In March, 1904, she began to undergo treatment with intravenous injections of water extract filtrate, which continued at intervals of twice a month until July, 1904. No precipitins were at any time demonstrable in her serum. In October, 1905, a dose of five hundred milligrams of old tuberculin No. 30 failed to elicit a reaction. A month later (Nov. 14, 1905), a virulent inoculation (intravenous) of twenty milligrams of culture "H 37" did not occasion a rise in temperature. From November, 1906, until the beginning of the present experiments she had injections of emulsion "T.C.R." No. 1 about twice a month. She has had no febrile reaction since October, 1903, except on Dec. 29, 1908, when she received an intravenous injection of an emulsion of B. tuberculosis culture, "H 37" (dried net weight = .06 Gen.).

It was thought advisable to repeat Yamanouchi's experiments with the serum of this cow. It was conceivable that its serum might possess specific properties (anti-bodies, etc.), analogous to those of the serum of tuberculous human individuals. The technic employed was essentially similar to that described by Yamanouchi. Young rabbits received intraperitoneal doses of the cow serum; and, twenty-four hours later, intravenous doses of a bacillary emulsion or extract emulsion. In some cases, if no symptoms followed the twenty-four-hour injection, the dose was repeated forty-eight hours after the introduction of horse serum. Most of the rabbits thus treated showed symptoms of one kind or another after the toxic injections. To control the results of the injections of this series of animals, a certain number of

rabbits were treated with extract emulsion after having received previous injections of the serum of normal cows (tuberculin tested). To control the technic further, a few rabbits received intravenous injections of extract emulsion without previous treatment with cow serum.

From the very beginning of the experiment it was observed that animals which had received similar previous treatment with cow serum differed rather widely in their response to the toxic injection, while the symptoms of animals that had been differently treated beforehand were identical after the toxic injection. This variability of toxic symptoms made it desirable to establish some criterion of what should and what should not be considered anaphylaxis.

Symptoms as described for the guinea-pig after injections of horse serum by Rosenau and Anderson[2] — scratching the mouth, coughing, spasmodic irregular breathing, paresis, convulsions and death — are well known and sufficiently characteristic. As a rule they appear very early: in from ten to fifteen minutes in Rosenau and Anderson's experiments; in from less than a minute to three minutes in Baldwin's[3] experiments where the antigen, extract emulsion, is introduced through the optic foramen. In the main, Yamanouchi's description of anaphylactic rabbits closely follows these authors. After the toxic dose the rabbits rapidly became dyspneic and suffered muscular weakness and paresis, a condition that was quickly followed by convulsions and death.

Such, in general, is the characteristic reaction, as it has been described, to a toxic injection of protein (serum protein, egg protein, etc.). Other more refined criteria of the phenomenon have been advanced. Biedl and Kraus[4] consider a fall in blood pressure to be the essential and primary feature of the condition and attribute all other symptoms as secondary to and dependent upon it. They mention incoagulability of the blood and an increase of the lymphocytes (at the expense of the polymorphonuclears) and blood platelets as accompanying the attack. Richet[5] and Arthus[6] also

observe lowering the blood pressure, but Richet does not agree that incoagulability of the blood is a frequent occurrence. Death does not necessarily complete the symptom complex. Animals may recover after symptoms have been either mild or severe.

While the so-called typical or acute anaphylaxis has been that in which the irritative phenomena perhaps as a rule predominate, it is becoming more and more customary to designate as anaphylaxis any symptoms whatever of toxicity that follow the toxic injection of a specific antigen. Such symptoms may be immediate, or they may be very, very gradual. They may be nothing more than slight weakness or labored respiration or dizziness. In a great many cases, without preliminary symptoms of restlessness or stimulation, the animal may slowly sink into a state of coma from which it does not recover; or, the characteristic dyspnea, the paresis, the convulsions may be absent; and immediately after the toxic injection the animal falls on its side and dies in a few minutes in a state of collapse. Sometimes no symptoms are apparent for hours after the toxic injection, yet the animal is found dead in its cage the next morning.

We have, therefore, several different types of reaction to the toxic injection that may be broadly classified as acute and gradual. In the acute type, the irritative symptoms, — the dyspnea, convulsions, voiding of urine and feces, restlessness, etc., — are generally seen. Both types are undoubtedly instances of hypersusceptibility to a specific antigen, but the anaphylactic reaction that is perhaps most widely known as typical or characteristic is the one that we have called acute.

Practically all of Yamanouchi's positive cases are of the acute type: only four or five are gradual reactions. In the following tables, for the sake of accuracy and completeness, the acute reactions will be designated as typical anaphylaxis, while the symptoms of all those cases where the response lacked definite irritative phenomena, but came on more gradually, will be given in detail.

The first series of experiments, brought together in Table 1, includes those animals which received preliminary treatment with the serum of Cow No. 5. Sixteen rabbits were intraperitoneally injected with this serum; and, twenty-four hours later, intravenously injected with bacillary extract emulsion. In a few instances the toxic injection of extract emulsion was repeated forty-eight hours after the introduction of serum. In all of the following tables, the caption, "first injection," means the intraperitoneal injection of serum; "first toxic injection," the first intravenous injection of extract emulsion; and "second toxic injection," the second intravenous injection of extract emulsion.

TABLE I.

Transference of serum of Cow No. 5 to rabbits.

Number of Animal.	Weight in Grams.	First Injection.	First Toxic Injection.	Clinical Symptoms.	Second Toxic Injection.	Clinical Symptoms.	Autopsy.
4 . . .	860	8 cc.	.5 cc. E.E. 152*	Typical anaphylaxis; gradual recovery.	5 cc. E.E. 152	Collapse; coma dyspnea; death in 3 minutes.	No lesions.
5 . . .	750	7 "	4.5 " " "	No symptoms.	1 " " "	Slow collapse; death in 15 minutes.	No lesions.
8 . . .	420	5 "	. . . . .	. . . . .	.5 " " "	No symptoms at first; dyspnea 4 hours after injection; recovery.	No lesions.
9 . . .	590	5 "	3 cc. E.E. 154X.	Typical anaphylaxis; death in 3 minutes.	. . . . .	. . . . .	Coccidiosis.
10 . . .	790	8 "	3 " " "	Typical anaphylaxis; death in 1½ minutes.	. . . . .	. . . . .	Coccidiosis.
14 . . .	400	5 "	4.5 " " "	Typical anaphylaxis; death in 5 minutes.	. . . . .	. . . . .	Pleural effusion.
15 . . .	330	5 "	3 " " "	No symptoms except weakness; death in 1 hour.	. . . . .	. . . . .	No lesions.
16 . . .	350	5 "	5 " " "	No symptoms except gradual collapse; death in 10 minutes.	. . . . .	. . . . .	Pleural effusion.
17 . . .	440	5 "	4 " " "	No symptoms.			
18 . . .	430	5 "	4.5 " " "	Shrill cries before end of injection; no other symptoms; found dead in cage 3 hours later.	. . . . .	. . . . .	No lesions.

26 . . .	470	5 "	5 " " "	Slight dyspnea; no definite symptoms.	5 cc. E.E. 154X.	Typical anaphylaxis; slow recovery.	Coccidiosis.
34 . . .	665	5 "	3 " " "	Typical anaphylaxis.	. . . . .	. . . . .	No lesions.
51 . . .	430	4 "	5 " " "	Typical anaphylaxis; death in 1 minute.	. . . . .	. . . . .	Coccidiosis.
58 . . .	610	6 "	5 " " 159	Typical anaphylaxis.	. . . . .	. . . . .	No lesions.
59 . . .	490	5 "	2.5 " " "	Slow collapse; died the following night.	. . . . .	. . . . .	No lesions.
60 . . .	920	8.5 "	6.5 " " "	No symptoms.	6 cc. E.E. 159	No symptoms.	

*See Appendix for description of bacillary preparations used.

This table shows that of the entire sixteen animals which received initial treatment with the serum of Cow No. 5, eight reacted acutely to the extract emulsion. The sensitive animals ranged in weight from four hundred and thirty grams (No. 51) to eight hundred and sixty grams (No. 8). A toxic dose of .5 cubic centimeter extract emulsion 152 elicited symptoms in a rabbit of eight hundred and sixty grams (No. 8), while it took repeated doses of five cubic centimeters extract emulsion 154X on successive days to bring out symptoms in a rabbit weighing four hundred and seventy grams (No. 26). It is noteworthy that Rabbit No. 4 was not rendered refractory to further doses of the specific antigen by its reaction to the first injection.

The remaining eight rabbits reacted rather variably to the toxic injections. Most of them went into a state of gradually increasing weakness and collapse that ended in death in from a few minutes to twelve hours. Only two of the animals had no symptoms whatever.

The procedure was repeated with the serum from three other cows. Of these, Cows "M" and "H" were healthy cows that had never been treated with tuberculin, but had been tuberculin tested about a year previously and had not reacted. Cow No. 16 had received .3 cubic centimeter of old tuberculin No. 130 subcutaneously on Nov. 10, 1905; .4 cubic centimeter on Nov. 16, 1905; .025 cubic centimeter of "T.C.R." No. 1 intravenously on Feb. 3, 1906; and .025 cubic centimeter of "T.C.R." No. 1 on Feb. 28, 1906. On Oct. 22, 1906, it failed to react to old tuberculin No. 130. It had therefore received no tuberculin for almost three years prior to the experiment and was presumably a healthy cow.

TABLE 2.

Transference of serum of Cow No. 16 to rabbits.

Number of Animal.	Weight in Grams.	First Injection.	Toxic Injection (Intravenous).	Clinical Symptoms.	Toxic Injection.	Clinical Symptoms.	Autopsy.
6 . . .	830	7 cc.	5 cc. E.E. 152	No symptoms.	.5 cc. E.E. 152	Typical anaphylaxis; death in 3 minutes.	No lesions.
7 . . .	860	8 "	2 " " "	Gradual collapse; death in 10 minutes; no typical symptoms.	. . . . .	. . . . .	No lesions.
11 . . .	520	5 "	5 cc. B.E. 154X.	No symptoms noted; put back in cage 2 hours later; found dead next morning.	. . . . .	. . . . .	Coccidiosis.
35 . . .	665	5 "	3 " " "	No symptoms.	. . . . .	. . . . .	Coccidiosis.
38 . . .	450	5 "	1.25 cc. B.E. 154X.	Typical anaphylaxis; death in 3 minutes.	. . . . .	. . . . .	No lesions.
52 . . .	300	4 "	. . . . .	. . . . .	5 cc. B.E. 154X.	No symptoms noted; found dead in cage 6 hours later.	Coccidiosis.
61 . . .	610	6 "	5 cc. B.E. 159	No symptoms.	5 " " 150	No symptoms.	
62 . . .	870	8.5 "	5 " " "	Typical anaphylaxis; death in 1½ minutes.	. . . . .	. . . . .	No lesions.
3 . . .	580	5 "	4 " " "	No symptoms.	5 cc. B.E. 159	No symptoms after injection; died 2 days later.	No lesions.

The results of the experiments in this series of animals resemble closely those obtained on Series 1. A certain proportion had the characteristic symptoms of typical, acute anaphylaxis, a number had the symptoms of weakness or collapse, while several did not react at all to the toxic injections. The two series were therefore almost identical in their results, although the cows that had furnished the sensitizing serum had received widely different treatment. It is true that both had been given the bacillary products, but it was hardly to be expected that the serum of an animal three years from the last injection would sensitize to the specific antigen as well as that of an animal that had been regularly treated during that time. Nevertheless the results pointed to the conclusion that Cow No. 16 retained its sensitiveness to the antigen for three years; that this sensitiveness might be transferred; and that, when transferred, it conferred as strong sensitiveness upon the rabbit as did the serum of Cow No. 5.

This conclusion was at the time further supported by the outcome of the experiments included in Table 3. This table represents those rabbits which after an initial injection of the serum of normal cows "H" and "M" were given the extract emulsion.

TABLE 3.

Serum of cows "H" and "M" transferred to rabbits.

No. of Animal.	Weight in Grams.	First Injection.	First Toxic Injection.	Clinical Symptoms.	Second Toxic Injection.	Clinical Symptoms.	Autopsy.
45	350	5 cc. H.	2.5 cc. E.E. 154X.	No symptoms.	3 cc. E.E. 154X.	Slow collapse; died the following night.	No lesions.
46	430	5 " "	2.5 " " "	" "			
53	730	6 " M.	5 " " "	" "	5 " " "	No symptoms.	
54	560	6 " "	2.5 " " "	" "	5 " " "	No symptoms.	
55	550	6 " "	5 " " "	" "	4 " " "	No symptoms.	
56	410	5 " "	4 " " "	" "			

The fact that practically none of these control animals reacted to the toxic doses (only Rabbit No. 45 showed symptoms after the second toxic injection) strongly indicated that the serum of treated cows acquired properties of reaction to the specific proteins of the tubercle bacillus that were not possessed by the serum of normal cows. This inference would have been compelling had it not been for the results of another series of control experiments that were undertaken from time to time during the investigation. The method of controlling the work further was to inject with extract emulsion young rabbits that had received no preliminary serum treatment. The rabbits were of the same stock and of the same average weight as those employed in the serum series. They were given the same preparations of bacillary products.

The result in a number of instances was rather surprising. Several of these rabbits developed symptoms that could not be distinguished from those of anaphylaxis. At any rate, if they had been sensitized beforehand with the specific antigen it is fair to assume that they would have been considered anaphylactic. The fact, however, that none of the rabbits had had a sensitizing dose and that so large a proportion of them succumbed to the extract emulsion precludes the possibility of considering them instances of congenital anaphylaxis or hypersusceptibility. Their "anaphylaxis-like" symptoms therefore must be due to something else. In Table 4 these symptoms are given in detail.

TABLE 4.

Intravenous injections with extract emulsion of rabbits that had received no previous treatment.

Number of Animal.	Weight in Grams.	First Injection.	Clinical Symptoms.	Second Injection.	Clinical Symptoms.	Autopsy.
42	360	4 cc. E.E. 154X.	Gradual collapse; death in 1 hour.	. . . . .	. . . . .	No lesions.
48	350	2 " " "	No symptoms.	3 cc. B.E. 154X.	Shrill cries; animal lay on side and became unconscious; death in 15 minutes.	No lesions.
64	700	5 " " 159	Gradual collapse; no convulsions or characteristic dyspnea; death in 10 minutes.	. . . . .	. . . . .	No lesions.
65	540	5 " " "	Immediate convulsions; respiratory spasm; death in 2 minutes.	. . . . .	. . . . .	Epicardial hemorrhages.
66	860	6 " " "	Immediate convulsions; respiratory spasm; death in 2 minutes.	. . . . .	. . . . .	No lesions.
69	900	9 " " "	No symptoms.			
28	430	5 " " 154X.	Rapid collapse and weakness; death in few minutes.	. . . . .	. . . . .	No lesions.
29	550	5 " " "	No symptoms.	. . . . .	. . . . .	Extensive coccidiosis.
70	650	3 " " 161	Respiratory embarrassment; fell over on side; unconscious; convulsions; death in 5 minutes.			

We see, therefore, that seven of the nine untreated rabbits responded to intravenous doses of extract emulsion exactly as did those treated with the serum of Cows No. 5 and 16. The most probable explanation of this occurrence is that young rabbits do not very well bear handling of this kind. In the Saranac Laboratory rabbits have time and again been thus treated in the course of routine work and have stood the injection without untoward effect. But such animals have usually been well-grown hares, which are generally quite resistant to the preparations of the killed tubercle bacillus. If, however, the rabbit is young and small, it seems to be very susceptible to intravenous injections, and a certain proportion is likely to succumb.

In order to determine exactly what symptoms a young rabbit with acquired sensitiveness to the bacillary products would exhibit, another set of experiments was performed. Young rabbits were given intraperitoneal doses of extract emulsion and water extract, and, twenty to thirty days later, intravenous doses of extract emulsion. The purpose of the experiments was to compare the symptoms that had been elicited in the above described series of rabbits with those of animals that were known to be sensitized and whose toxic manifestations ought therefore to be typically anaphylactic.

Experiment A: VI/4/09. Rabbit No. 22, 385 grams. Injected intraperitoneally with 5 cubic centimeters Ext. Emul. 154X.
VII/3/09. Twenty-nine days after sensitization. Injected intravenously with 1 cubic centimeter Ext. Emul. 154X.
Result: Shrill cries, immediate respiratory spasm, paresis, convulsions. Death in two minutes.
Autopsy: No lesions.

Experiment B: VI/4/09. Rabbit No. 23, 435 grams. Injected intraperitoneally with 5 cubic centimeters Ext. Emul. 154X.
VII/3/09. Twenty-nine days after sensitization. Injected intravenously with 3 cubic centimeters Ext. Emul. 154X.
Result: Animal lay on side and rapidly lost consciousness. Death in 2 minutes. No irritative symptoms.
Autopsy: No lesions.

Experiment C: XII/2/09. Rabbit No. 71, 420 grams. Injected intraperitoneally with 2.5 cubic centimeters W.E. 162.
XII/22/09. Twenty days after sensitization. Injected intravenously with 1.5 cubic centimeters Ext. Emul. 162.

Result: Shrill cries. Dyspnea. Rapid collapse. Death in 2 minutes.
Autopsy: Slight coccidiosis of liver. No other lesions.

These should be instances of true anaphylaxis, for, following an incubation period during which sensitiveness was being acquired, the animals received the toxic dose of the same antigen as had sensitized them. The symptoms cannot be distinguished from those which occurred in the other series of experiments. To explain the similarity of symptoms with those of Table 4, two views may be advanced: either that the animals of Series 4 (and therefore a certain proportion of all young rabbits) are naturally or congenitally sensitive to extract emulsion (employed intravenously); or, that where in young rabbits the symptoms of toxicity follow the intravenous introduction of extract emulsion they cannot be clinically differentiated from the manifestations of anaphylaxis. The latter inference is the more plausible of the two, for while instances of congenital sensitiveness to an antigen are not very uncommon they can by no means be conceived to be so frequent as Table 4 would show.

If animals that are not sensitive respond to the toxic doses of an antigen with anaphylactic symptoms, their value as experimental animals in this connection is obviously nil. Yamanouchi lays stress on the employment of animals that weigh from five hundred to seven hundred grams. He notes that larger animals are refractory or are uncertain in their response to the toxic dose. Our different series indicate that animals weighing from three hundred and fifty to eight hundred and sixty grams will show symptoms irrespective of their being treated beforehand with a sensitizing dose. If, then, as Yamanouchi maintains, the larger animals are unsuitable for use it would follow that no rabbits should be used for the diagnostic test. Their use would lead to essential errors of interpretation of the symptoms.

Since the method is condemned by the control animals it is plain that no conclusion can be drawn from the experiments on the rabbits that were treated with the cow serum.

Several apparent contradictions exist in the tables. The question might be asked why all but one of the rabbits that had been treated with normal cow's serum bore the toxic injection without effect while seven of the nine untreated rabbits succumbed. The answer to this query must be that only a certain proportion of the experimental animals will show such toxic symptoms. To determine how large a proportion would require sets of experiments comprising large numbers of animals. The very fact that these discrepancies in results occur simply serves to emphasize the uncertainty of the method. One might inject a comparatively large series of these animals without producing any effect; but, on the other hand, the next series might show a large proportion of fatal cases.

At the Saranac Laboratory experiments are now going on that bear some relation to this matter of the toxicity of intravenous injections of bacillary products to young rabbits. It has been found that pure water extract filtrate causes no symptoms. No rabbit has yet shown toxicity if it received through the ear vein relatively large doses of the filtrate. This fact holds good not only for those rabbits that have been untreated beforehand, but also even for those that have been previously sensitized with the filtrate. On the other hand, such animals almost invariably respond to toxic injections of the extract emulsion. In this connection it is significant that Yamanouchi records that the best working preparation was an extract emulsion, while the other preparations were uncertain in their effects.

This observation opens up a promising opportunity for the study of the mechanism of the toxic action of bacillary products, and, perhaps, of anaphylaxis itself. It is a rather remarkable fact that if a filtrate carries with it the dead, crushed bodies of the bacilli it is very toxic, while if pure and freed from them it has no potency whatever in non-tuberculous animals — not even enough to arouse a response in animals previously sensitized to the protein. We have never been able to demonstrate pulmonary emboli in those rabbits that succumbed to the extract emulsion. Work is now proceeding along the

line of several different hypotheses in an effort to clear up this matter. One is that the crushed bacilli carry relatively large amounts of protein and that these protein-bearing bacilli when introduced into the blood stream find their way into the vessels of the medulla, where on account of their bulk they remain lodged or proceed slowly. This event would bring a sufficient quantity of protein into more intimate and more prolonged contact with the medullary cells, than would be the case when the filtrate alone was sweeping through the capillaries.

Another possible occurrence might be capillary embolism of the bulb. Again, it is conceivable that the protein clings to or is loosely bound with the fatty constituents that make up so large a part of the tubercle bacillus, and that if loosely bound to the fats it is for that reason more easily assimilable for the brain cells. Kyes'[7] discovery of cobra-lecithid — the union of two innocuous substances, a protein and a fatty, to form a hemolysin — is an instance of the power that fats have of rendering proteins toxic. Studies along these lines are now in progress and their results will be reported in subsequent communications.

CONCLUSIONS.

1. Young rabbits exhibit symptoms of toxicity when injected *intravenously* with extract emulsions of the tubercle bacillus.*

2. These symptoms cannot be distinguished clinically from those of anaphylaxis.

3. The Yamanouchi test is therefore likely to be too uncertain in its results to be of diagnostic value.

[I am deeply grateful to Dr. E. R. Baldwin for his valuable assistance throughout this work, which was prosecuted under his direction and guidance.]

* In a footnote (Handbuch der Technik und Methodik der Immunitätsforschung — Kraus and Levaditi, vol. ii, p. 888), Kraus states that in rabbits the symptoms described by Yamanouchi depend on the initial toxicity of the tuberculin employed. After preliminary treatment with normal horse serum a toxic tuberculin will elicit symptoms in rabbits, while other tuberculins cause no symptoms in rabbits that have been treated with serum of tuberculous human beings.

REFERENCES.

1. Yamanouchi, T. Wiener Klinisch Wochenschrift, 1909, No. 47, 1623.
2. Rosenau and Anderson. Government Hygienic Laboratory, 1906, Bulletin No. 29, 14.
3. Baldwin, E. R. Journal of Medical Research, xxii, 2, p. 189.
4. Biedl, A., and Kraus, R. Wiener Klinische Wochenschrift, 1909, No. 11, 363.
5. Richet, Ch. La Presse Medicále, 1909, No. 28, 249.
6. Arthus, M. La Presse Medicále, 1909, No. 35, 305.
7. Kyes, P. Berliner Klinische Wochenschrift, 1903, No. 42, 956.

(*c.*) NOTE ON THE ACQUIRED GENERAL SENSITIVENESS OF NORMAL GUINEA-PIGS TO TUBERCULO-PROTEIN AS A RESULT OF PRELIMINARY INTRADERMIC INJECTIONS.*

A. K. Krause, M.D.

In the course of a series of experiments that I was making in an investigation of the acquired sensitiveness of guinea-pigs to the protein of the tubercle bacillus, I had occasion to test several animals which had been previously treated with the intradermic test (Mantoux) when they had served as controls to the same test on tuberculous animals. The experimental details follow:

Jan. 22, 1910: Injected guinea-pigs, Exp. 286, Nos. 1, 4, and 5, weighing 490, 350, and 335 grams respectively, each with .1 cubic centimeter W.E. 163 intradermically. These were normal animals.

January 23 and 24: Except for slight initial reddening and papule (traumatic) which soon disappeared there was no reaction. Result: negative.

January 24: Repeated the intradermic injection of the above animals with .1 cubic centimeter W.E. 163.

January 25: Result: negative.

Toxic Injections.

Feb. 7, 1910: Fourteen days after last test.

Injected, post-orbitally, No. 1 with .75 cubic centimeter extract emulsion 161. There immediately ensued moderate but typical anaphylactic symptoms that were unusually prolonged: spasmodic cough, respiratory spasm, dizziness, and "bucking." The animal made a gradual recovery.

Injected, post-orbitally, No. 4 with .75 cubic centimeter extract emulsion 161. Result: moderate, prolonged symptoms as in No. 1; gradua recovery.

March 5, 1910: Forty days after last intradermic test.

Injected, post-orbitally, No. 5 with .75 cubic centimeter extract emulsion 161. Result: very fulminant anaphylaxis with death in extreme inspiratory spasm in three minutes. Autopsy: tremendously insufflated lungs, epicardial hemorrhages, large hemorrhage into parietal peritoneum, general dilatation of splanchnic blood vessels; no other lesions.

* Received for publication March 7, 1910.

Summary of experiments. — Two normal guinea-pigs, that had received intradermically .2 cubic centimeter of a watery extract filtrate in two doses of .1 cubic centimeter each, at an interval of two days, exhibited marked general sensitiveness to the protein when injected post-orbitally after an incubation period of fourteen days.

One normal guinea-pig subjected to preliminary treatment similar to the above, showed extreme general sensitiveness after an incubation period of forty days.

The above observations are suggestive and possess possibly more than theoretical significance. In the present state of our knowledge we do not know how closely the mechanism of the tuberculin reaction in tuberculous animals is related to that of the phenomena attendant upon acquired sensitiveness to the bacillary protein in non-tuberculous animals. Both processes have been quite thoroughly studied in the guinea-pig, but have as yet not been brought into harmony with each other. The problem of the tuberculin reaction in man has also been very fully investigated, but the question of acquired tuberculin hypersensitiveness in non-tuberculous human beings has not been systematically looked into. Nevertheless the experiments that I have cited suggest the point, whether, given a proper incubation interval, the various diagnostic cutaneous reactions as practised on man may not in some instances sensitize the non-tuberculous subject to subsequent general tests; *e.g.*, the subcutaneous test.

[*Note:* Both the practical and theoretical consideration of the problem of acquired sensitiveness in its relation to the tuberculin reaction have been very fully discussed in the paper by Dr. E. R. Baldwin.]

II. THE EFFECT OF THE ADMINISTRATION OF PREPARATIONS OF TUBERCULOUS LYMPH GLANDS ON EXPERIMENTAL TUBERCULOSIS.*

E. L. Trudeau, M.D., and A. K. Krause, M.D.

About a year ago Livierato[1] published the results of a series of experiments that he had performed on guinea-pigs, in which he sought, by means of injections of tuberculous lymph gland extracts to immunize these animals against experimental infection with the tubercle bacillus. From his investigations he concluded that these extracts conferred a certain degree of protection upon the inoculated animals. At the time of the appearance of Livierato's paper we were engaged in practically the same work. Our results have not agreed with his. On this account we have thought it worth while to give an account of our studies, prefacing the experimental details with a summary of the more important contributions that the past two decades have added to this branch of our knowledge of the processes of immunity against tuberculous infection.

It was a very early observation that tubercle bacilli that had been recovered from lymphatic glands were less virulent than those obtained from diseased foci of other organs. In a paper published in 1886 Arloing[2] relates differences that he obtained when he inoculated guinea-pigs and rabbits with the bacilli from scrofulous products on the one hand and from other tuberculous lesions on the other. He concluded that the former would infect guinea-pigs but not rabbits, that he could not raise the virulence of the scrofulous bacilli by continuous passage through guinea-pigs while he could increase that of bacilli obtained elsewhere, and that therefore the former had departed further from their original virulence than had those from other tuberculous organs. Arloing's work was later confirmed by Moore,[3] who inoculated guinea-pigs and rabbits with pieces of lymph nodes from twenty-two

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cases of scrofulous lymphadenitis and, when able to infect the first but not the second species of animal, concluded that bacilli from scrofulous glands were less virulent than those from expectoration. Baumgarten [4] promptly pointed out that the reasoning of both Arloing and Moore was fallacious and based on incomplete experimentation. He contended that rabbits could be infected with scrofulous bacilli, and that bacilli not only from scrofulous glands but from the products of other human tuberculosis as well, are often so diminished in virulence that they will not even infect guinea-pigs, while in other cases those from scrofulous glands are virulent enough to infect rabbits fatally.

Directly related to the foregoing work were the investigations of Pizzini [5] on the infectiousness of lymph glands that were morphologically intact. He inoculated guinea-pigs with glands from forty human beings that had died of acute disease or accident. Although he could determine no gross or microscopic evidences of tuberculosis in any of the glands, he found that forty-two per cent of his inoculated animals became tuberculous. Apparently normal bronchial glands were particularly infectious, though in a few instances cervical and mesenteric glands produced the disease. Here was direct evidence that to a certain degree at least lymph glands could resist infection by virulent tubercle bacilli and were to that extent immune. It was confirmed later experimentally by Weichselbaum and Bartel,[6] who after inoculating material from tonsils and regionary lymph glands concluded that the tubercle bacillus may remain viable for a long time without causing any specific tuberculous changes in these organs. Pizzini obtained his glands from autopsy on adults, while those of Weichselbaum and Bartel were from children. Kälble,[7] Macfadyen and MacConkey,[8] and Harbitz [9] all report positive results from inoculating guinea-pigs with apparently normal glands, the sources of which were for the most part infants and children.

In his studies on the behavior of the lymphatic system towards microörganisms Perez [10] approached the problem of glandular protective action directly. He recovered tubercle

bacilli in pure culture from the lymphatic glands of inoculated animals and with these infected other animals. Through several successive passages of such bacilli, obtained always from the lymph glands of infected animals, he got infections that grew continually milder. After the third and sometimes after the second passage such lymph gland cultures caused only mild infections. Animals that were killed ten or more months after inoculation showed only swelling of the glands and calcified tubercles of the various organs.

Several years later Manfredi[11] and his assistants published an extremely laborious and complete series of experiments the object of which was to define the importance of the lymph ganglia in the processes of infection and immunity. Paying particular attention to the tubercle bacillus, they concluded that the lymph nodes protect the organism against bacteria in a three-fold manner: (1), by a process of filtration in which the further spread of the bacteria is checked; (2), by an enervating action by which in adenoid tissue the virulence of the bacteria is diminished; (3), by an immunizing action that the organism attains by virtue of the first two processes. They, however, emphasized the point that, if in the struggle between bacteria and lymph glands the function of the latter became markedly impaired, the glands might become foci of real danger to the rest of the body, harboring as they would large numbers of virulent microörganisms One of the most interesting results of their researches was the apparent demonstration of the occurrence of a specific immunizing substance in tuberculous lymph glands. An emulsion made from the gland of a goat immunized by repeated subcutaneous injections of tubercle bacilli was injected into a guinea-pig simultaneously with virulent bacilli. After forty-seven days the guinea-pig showed only a single, caseous, subcutaneous nodule at the site of inoculation while a control animal had generalized tuberculosis. The fact that only one instance of active immunization was cited, detracted somewhat from the value of the observation and so far as can be ascertained no similar experiments were subsequently made by these authors.

Manfredi and Frisco [12] continued experimental work on the lymph glands as protective agents against tuberculosis. By means of endolymphatic injections of small doses of bacillus tuberculosis they determined: (1), that in the glands there may exist a condition of latent tuberculosis when the bacilli may be present without the occurrence of the corresponding tissue changes; (2), that there may occur a tuberculosis of characteristic appearance that is confined to the lymphatic glands; (3), that a tuberculosis may result that is first localized in the glandular system and then spread to other organs and become general. It is therefore clear, they conclude, that in many cases the glands exercise a specific action for their own protection as well as for the protection of the entire organism. This defensive action they believed due in part to the anatomical structure of the glands, which by filtering out the bacilli stopped them from further assault on the organism, and in part to a power that the glands possessed of dissolving and killing the bacteria.

The most assiduous worker in the field of lymph gland immunity and protection against tuberculous infection has been Bartel, who in the last five years has published many papers on the subject which may here be briefly summarized. One of the author's first papers [13] pointed out that, before leading to specific tubercle formation and caseation, lymph gland tuberculosis goes through a preliminary stage of non-specific small cell hyperplasia, — a latent stage that may exist for one hundred days and probably much longer. Whether the lymphocytes act in inhibiting the virulence or activity of the microörganism he was not then prepared to say. Pursuing his studies further, Bartel [14] concluded that in a slightly susceptible animal the lymph glands can convert virulent into avirulent bacilli. A year later Bartel and Neumann [15] announced that they had obtained diminished virulence by soaking tubercle bacilli in emulsions of normal mesenteric glands. Such bacilli gave rise in the guinea-pig to cirrhotic, healing lesions in the liver and the infected animals lived longer than control animals. They assumed therefore the existence of a specific devitalizing substance in normal glands. It is of interest to note here that Neumann

and Wittgenstein[16] in a paper published at about the same time found that this property was not possessed by lymph glands alone, but could be assigned as well to the spleen, liver, and ovary, while the lung and blood exhibited no effect on the virulence of the bacteria.

In later papers, Bartel[17, 18, 19] confined his experiments to the inoculation of susceptible animals (guinea-pigs) with tubercle bacilli that have been soaked in decoctions of lymph glands and of various other organs. After inoculations of bacilli that have been soaked in different organs he notes the resultant infection taking a favorable course in the guinea-pig. Bacilli suspended in organs of healthy animals at 37° are after a time viable but avirulent. After receiving inoculations of such bacteria animals show no signs of manifest tuberculosis, but their lymphatic glands undergo more or less hyperplasia. The best results are always obtained from extracts of lymph glands.

By the use of extracts of tuberculous lymph glands (Bartel had worked with normal extracts), Rodet[20, 21] practised perhaps the most active attempts at lymph gland immunization of which we have any record. He made emulsions of guinea-pig lymph glands that were in more or less advanced stages of experimental tuberculosis, macerating the glands from two or three animals in one hundred cubic centimeters of sterile water. He preserved this emulsion with thymol and used it only after all the contained bacilli were dead. One series of animals he treated with this emulsion before infection; another series, after infection with virulent bacilli. The extract in one lot of the animals was administered alone, in another lot it was given with the addition of fresh horse or goat serum. It was applied every two or three days until the animals had received thirty injections. The results were entirely negative: the injections of gland extract neither prevented nor cured the infection in the least. All the animals died of generalized tuberculosis of the same stage and nature as exhibited in the control animals.

Livierato's[22] procedure was essentially similar to Rodet's, but resulted differently. By inoculating a series of animals

with tuberculous material he obtained a mild infection, especially of the glands. He made an extract of the glands, sterilized it and preserved it with chloroform in the ice-chest. With this sterile material he treated his experimental animals and got a prolonged mild infection. The result was therefore an apparent immunizing effect of the tuberculous gland extract.

Our own experiments proceeded in general along the lines followed by Rodet and Livierato with some minor though fundamental changes in technic. The experiments fall into four series: two with homologous gland extracts, and two with extracts of human lymph glands. In the first series we were not certain whether or not the extracts contained living bacilli; in the other three series they were undoubtedly bacilli free.

SERIES I.*

Source and preparation of gland material. — Twenty-four guinea-pigs (Exp. 266, Nos. 1 to 24) on Aug. 7, 1908, were inoculated subcutaneously each with .25 milligram R I culture. Beginning Nov. 4, 1908, they were killed, two or three at a time, at intervals of about two weeks. The infection of all these animals (Exp. 266) was a uniformly very mild one. Autopsy generally showed a lymphatic hyperplasia of varying degree, but never caseation of the glands. There were local cold abscesses in those animals that were first sacrificed, but as a rule none in those that were allowed to live longer. The infection apparently tended spontaneously towards healing, for one guinea-pig, No. 17, that was allowed to live for almost a year showed no evidences of tuberculosis; while another, No. 16, exhibited only slight hyperplasia of the right inguinal glands. The glands were removed, crushed fine and used immediately to treat the experimental animals.

Treatment of experimental animals with lymph gland material before virulent inoculation. — Ten guinea-pigs (Exp. 266 A, Nos. 1 to 5 and 11 to 15) were subcutaneously injected on November 4, November 19, and December 7 with fresh emulsions of the crushed lymph glands. Each was given 1.5 cubic centimeters of the emulsion at each of the three injections. Each accordingly received three doses of the gland material before inoculation.

Inoculation of animals. — Dec. 7, 1908: Emulsion of B. tuberculosis, serum culture H37, made as follows: A spadeful of the culture was rubbed up in a mortar with a minimal amount of physiological salt solution until

* Our acknowledgments are due to Dr. J. Woods Price and Dr. E. R. Baldwin who made the inoculations and performed the autopsies of the animals of Series 1.

the emulsion was fairly homogenous. This was then diluted and centrifugated for fifteen minutes, further diluted and filtered until the emulsion was free from large clumps and contained roughly an average of one bacillus to every two fields of the immersion lens. Each guinea-pig, Exp. 266 A, Nos. 1 to 5 and 11 to 15, received .1 cubic centimeter of this emulsion subcutaneously in the right groin. Normal control animals, Exp. 266A, Nos. 21 to 25, were similarly inoculated.

Treatment of experimental animals with lymph gland material after inoculation. — Guinea-pigs, Exp. 266 A, Nos. 1 to 5 and 11 to 15, were injected on Dec. 21, 1908, Jan. 4 and Jan. 18, 1909, each with 1.5 cubic centimeters of crushed lymph gland emulsion, at every injection. On Jan. 18, 1909, treatment was discontinued and the animals were allowed to live for five or six weeks. Together with the control animals they were killed on Feb. 25 and 26, 1909.

Result. — The differences in degree of infection between the treated animals and the controls were not sufficient to indicate that the gland emulsions conferred any definite protection. It is true that in the greater proportion of the controls the disease had advanced as far as caseous tuberculosis of the liver, but in so small a number of animals this occurrence is well within the limits of variation in individual resistance. The lesions in general were no less uniform than one would expect after subjecting fifteen guinea-pigs to inoculation under identical conditions. The regional glands were always more or less hyperplastic and in most instances caseous, — caseous in the treated as well as in the control animals. In all the animals but one treated guinea-pig the disease had reached the spleen, where the lesion encountered ranged from beginning infiltration to massive caseous tuberculosis, irrespective of the condition of the animal prior to inoculation. One treated and one control guinea-pig showed pulmonary involvement. There was no instance of generalized tuberculosis.

Discussion. — The conditions of this experiment corresponded closely to that of Livierato's, except that it was very probable that we were using glandular material that contained tubercle bacilli. In every case we subjected the emulsions to microscopic examination and were unable to demonstrate bacteria. Such examination, however, would

not rule out the possibility of their being present. As a matter of fact, we assumed that they did exist in our emulsions. Such bacteria would be of our R I culture, which is of very slight virulence for guinea-pigs and which has been demonstrated to confer upon guinea-pigs some immunity to subsequent virulent infection. If, therefore, they were present in our emulsions we rather expected that they might bestow a certain amount of protection upon the treated animals. We would think a priori that our non-sterilized material would exercise a more beneficial effect than Livierato's sterilized extracts.

SERIES 2.

As in Series 1 the material used in this experiment was obtained from homologous lymph glands. It differed from the former, however, in that it was passed through a Berkefeld filter and was therefore bacilli free. It was then preserved with toluol in the ice-chest.

Source and preparation of gland substance. — March 25, 1909: Twelve guinea-pigs, Exp. 276, Nos. 1 to 12, were inoculated subcutaneously each with .5 milligram of R I culture of B. tuberculosis. On June 11 and 12 these animals were killed. The lesions in all were very uniform, consisting only of local cold abscesses and hyperplastic, non-caseous, regionary glands.

The glands of all twelve guinea-pigs were removed. Their moist weight was 10 grams. They were rubbed up in a mortar with copper gauze and extracted with 100 cubic centimeters of physiological salt solution. The resultant extract emulsion was centrifugated and the supernatant fluid filtered twice through a Berkefeld filter. About 70 cubic centimeters of filtrate was recovered, which was treated with a layer of toluol and set aside for immunizing experiments. The filtrate, treated with heat and dilute acetic acid, precipitated a slight cloud. Two guinea-pigs, Exp. 276, Nos. 23 and 24, were injected subcutaneously, each with 2.5 cubic centimeters of the filtrate (June 14, 1909). These were killed four months later and were found to be free from tuberculosis. The absence of tubercle bacilli from our extract was thus assured.

Treatment of experimental animals with lymph gland material before inoculation. — Five guinea-pigs, Exp. 276, Nos. 16 to 20, received 1 cubic centimeter of the extract subcutaneously at three different times — June 14, June 18, and June 22, 1909.

Inoculation of animals. — June 28, 1909, six days after last injection of gland extract. The emulsion of tubercle bacilli from a culture of H37

was prepared in the same manner as that used for inoculation in Series 1. Smears showed one bacillus to every two or three fields. There were no large clumps. Guinea-pigs, Exp. 276, Nos. 16 to 20, as well as five controls, Nos. 25 to 29, were inoculated, each receiving .1 cubic centimeter of the emulsion subcutaneously in the right groin.

Result. — Aug. 23, 1909, fifty-six days after inoculation. All the animals were killed by puncturing the medulla. There was no particular difference of tuberculous involvement between the treated and the control animals. There was as a rule hyperplasia and caseation of the regional lymph glands and in some instances involvement of the spleen. There were no other lesions.

Discussion. — This set of experiments was very unsatisfactory because, on account of contamination of the extract, the animals received only three doses before inoculation and none subsequent to it. The extract, too, was very dilute, showing, as it did, only a trace of protein.

Series 3.

In this series the lymph gland extract was obtained from tuberculous human glands which were removed by operation from the axilla and which came to us through the kindness of Dr. Hodenpyl of New York. They were received in good condition and separated from the fat and areolar tissue. Many had caseous foci, some were merely hyperplastic, while others were apparently healthy. The whole mass of glands weighed twenty-two grams moist.

Preparation of gland material. — The entire lot of glands was washed rapidly in distilled water and then ground to a pulp in a mortar with copper gauze. This pulp was then digested with 220 cubic centimeters of sterile physiological salt solution. The material was centrifugated and the supernatant fluid passed through a Berkefeld filter into a sterile flask. A second filtration was then performed and the filtrate was finally put into sterile test-tubes in 5 cubic centimeter lots, preserved with toluol and kept in the ice-chest. Guinea-pigs, Exp. 279, Nos. 11 and 12, were injected subcutaneously each with 2.5 cubic centimeters of the extract (June 7, 1909). They died on August 31, 86 days afterwards, and were found to be free from tuberculosis.

Treatment of experimental animals before inoculation. — Guinea-pigs, Exp. 279, Nos. 1 to 10, received subcutaneous injections of 1 cubic centimeter each with the extract on June 10, 14, 18, and 22, 1909.

Inoculation of animals. — June 22, 1909: An emulsion of H37 culture was prepared as outlined under Series 1. Smears contained one bacillus to two fields. There were no large clumps. Guinea-pigs, Exp. 279, Nos. 1 to 10, and Controls, Nos. 13 to 22, were inoculated with this emulsion, each receiving .1 cubic centimeter subcutaneously in the right groin.

Treatment of experimental animals after inoculation. — On account of intercurrent affections the majority of both the treated and the control animals died very soon after the virulent inoculation. The cause of death in these animals so far as could be ascertained was probably an acute enteritis, which at the time carried off many of our laboratory guinea-pigs. However, we were successful in carrying three of the treated animals and five controls through the experiment. The three treated animals, Exp. 279, Nos. 2, 7, and 9, received three doses of the extract after the inoculation, — 1 cubic centimeter each on June 29, July 9, and July 14, 1909.

Result. — Aug. 23, 1909, sixty-two days after inoculation and forty days after the last injection of extract. The surviving treated and control animals were killed by puncture of the medulla on this day. The result was strikingly in favor of a slighter degree of infection in the treated as compared with the control guinea-pigs. In the treated animals the local abscess was slight or lacking altogether. There was almost uniformly only slight enlargement and caseation of the regional lymph glands; the involvement of the spleen was very slight in two cases out of three; there was no gross tuberculosis of the liver, lungs, or other glands; there was no generalized tuberculosis, and all the animals gained in weight. The controls, on the other hand, generally showed large or enormous local abscesses, greatly enlarged and caseous glands, advanced caseous spleens, miliary tuberculosis of the liver and lungs and generalized tuberculosis. Three of the five controls remained stationary in weight, while two lost weight. The accompanying table shows the changes more graphically:

TABLE.

Treated Animals.	Weight.	Abscess at Site of Inoculation.	Superficial Inguinal Glands.	Deep Inguinal Glands.	Iliac Glands.	Spleen.	Liver.	Lungs.	Cervical Glands.	Generalized Tuberculosis.
No. 2. . .	Increase 70.	O	Slight enlargement and caseation.	Slightly enlarged and caseous.	Slightly enlarged and caseous.	Very slight enlargement.	O	O	O	O
No. 7. . .	" 90.	Slight.	Slight enlargement and caseation.	Slightly enlarged and caseous.	Hyperplastic and fibrous.	Very slight enlargement.	O	O	O	O
No. 9. . .	" 35.	O	Slight enlargement and caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Slightly enlarged and nodular.	O	O	O	O
Control Animals.										
No. 15 . .	Stationary.	O	Great enlargement and widespread caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Slightly enlarged.	O	O	O	O
No. 16 . .	Decrease 60.	Enormous.	Great enlargement and widespread caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Greatly enlarged; massive caseous tuberculosis.	Miliary tuberculosis.	Miliary tuberculosis.	Enlarged and caseous.	+
No. 17 . .	Stationary.	"	Great enlargement and widespread caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Greatly enlarged; massive caseous tuberculosis.	Miliary tuberculosis.	Miliary tuberculosis.	Enlarged and caseous.	+
No. 18 . .	"	"	Great enlargement and widespread caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Greatly enlarged; massive caseous tuberculosis.	O	Miliary tuberculosis.	Enlarged and caseous.	+
No. 19 . .	Decrease 10.	Large.	Great enlargement and widespread caseation.	Slightly enlarged and caseous.	Enlarged and caseous.	Greatly enlarged; massive caseous tuberculosis.	Slight involvement.	Miliary tuberculosis.	Enlarged and caseous.	+

Discussion. — The result of this series of experiments was certainly suggestively in favor of the assumption that the tuberculous lymph glands contain an immunizing substance that might be actively employed. But, because so few of the original animals survived, the result could be interpreted only as suggestive and did not warrant the definite conclusion that the protective substance had been demonstrated with certainty. All investigators of experimental tuberculosis are familiar with the fact that very considerable differences of individual resistance occur even in that highly susceptible animal, the guinea-pig. They are accordingly careful to draw deductions of comparative grades and degrees of infection only when a very marked regularity of variation occurs between sets of animals that have been experimented upon under different conditions and when these series of animals include a sufficiently large number of individuals. The very fact that one and only one of our five control animals (No. 15) differed so widely at autopsy from the other controls and approached very closely the condition noted in the treated animals serves to illustrate our point. We therefore determined to repeat the conditions of this experiment in the hope that the result might bear out the suggestion of protection offered by Series 3.

Series 4.

Through the kindness of Dr. McCordick of Montreal we obtained seventy grams (moist weight) of tuberculous human glands that had been removed at operation. Most of these glands showed caseous foci. Freed from surrounding fat and connective tissue and washed rapidly in distilled water, they were prepared for use.

Preparation of gland material. — June 29, 1909: The glands were ground fine in a meat chopper and allowed to stand over night in 200 cubic centimeters of a mixture of equal parts of physiological salt solution and solution of sodium carbonate, .5 per cent, preserved with a thin layer of toluol in the ice-chest. The next morning the resultant emulsion was strained through muslin, centrifugated and passed through a Berkefeld filter into a sterile flask. About 130 cubic centimeters of clear fluid remained. Tests with heat and dilute acetic acid threw down a very

heavy precipitate. The strength of the original fluid was roughly about one part gland substance to 2.5 fluid. The filtrate was put up in sterile, paraffined test-tubes, 5 cubic centimeters to a tube, and each tube containing a slight layer of toluol. The tubes were kept in the ice-chest until Sept. 25, 1909, when we began the treatment of the guinea-pigs. After three months in the ice-chest the filtrate remained clear, uncontaminated and without sediment.

Treatment of experimental animals before inoculation. — Five guinea-pigs, Exp. 289, Nos. 1 to 5, received ten injections every three or four days for a month, as follows: each was injected subcutaneously with 1 cubic centimeter of the filtrate on Sept. 25, 28, and Oct. 1, 4, 7, 12, 16, 21, 23, and 26.

Inoculation of animals. — Oct. 28, 1909, two days after last treatment. An emulsion of H37 culture was made in the usual manner. Smears showed no clumps, but an average of one bacillus to the field or less. Guinea-pigs, Exp. 289, Nos. 1 to 5, and controls, 6 to 10, were inoculated, each subcutaneously with .1 cubic centimeter in the right groin.

Treatment of experimental animals after inoculation. — The guinea-pigs, Nos. 1 to 5, were injected seven times after inoculation, with 1 cubic centimeter of the filtrate on October 30 and November 4, 9, 13, 17, 22, and 29.

Result. — The treated and control guinea-pigs were killed by puncture on December 24, twenty-five days after the last treatment and fifty-seven days after inoculation. There was no difference of tuberculous involvement between the two sets of animals. Practically all the treated and control animals were suffering from advanced and widespread tuberculosis. The animal that was least affected was Control No. 7.

Discussion. — This was by far the most rigid test to which we had put our technic. The lymph gland extract was quite concentrated and would therefore be presumably more efficacious than the higher dilutions that we had previously used. Each animal received seventeen injections, ten before and seven after inoculation. Yet the result was disappointing and justified only one conclusion; namely, that material from tuberculous glands obtained by the ordinary methods of extraction exercise no preventive or curative influence on the course of experimental tuberculosis and do not in the least inhibit the development and spread of the bacteria within the body.

When we summarize the conditions and results of the four series of experiments we arrive at the following conclusions:

1. Guinea-pigs treated with material from homologous tuberculous glands are not immunized against the development or spread of experimental tuberculous injection. The gland material in this series probably contained tubercle bacilli.

2. Guinea-pigs treated with filtered extract of homologous tuberculous glands do not resist experimental tuberculosis. This extract was used in a comparatively fresh condition. The experiment was hardly a fair test because of the high dilution of the extract and the small number of doses that the animals received.

3. Guinea-pigs treated with filtered extract of tuberculous human glands showed an apparent resistance to the spread of tuberculous infection. As discussed in the text of this paper, this resistance was probably only apparent. The extract was from glands in various stages of diseases — hyperplastic and necrotic. Treatment was fairly thorough; and the use of the extract was begun immediately after preparation and continued for six weeks.

4. Guinea-pigs treated more thoroughly than those of Series 3 with filtered extract of tuberculous human glands acquired no immunity against infection from tuberculosis or its development. The extract was from glands that showed various grades of disease, from early to advanced. It was concentrated and its use was begun three months after its preparation.

It is of course possible that if an immunizing substance does occur in tuberculous glands, it was lost in the course of some of the manipulations of our technic. It might have disappeared or been greatly weakened in our filtrations; or, in the last series of experiments, by being allowed to stand three months before its use was begun. In these first attempts at obtaining a protective material we employed glands in all stages of tuberculosis. Perhaps it shall turn out that the disease in most of our material was too far advanced, and that antibodies, if present, occur most

abundantly in healing or slightly affected glands. We must ask ourselves, too, whether, in some of our experiments, we did not use our material in so dilute a form as to do away with its effectiveness.

Bearing on the question of the elaboration in tuberculous glands of antibodies against the bacillus, is a recent paper of Fontes,[23] who has performed in vitro experiments to demonstrate a specific bactericidal power inherent in such glands. He immersed given quantities of tubercle bacilli in given amounts of tuberculous gland emulsions, normal gland emulsions and physiological salt solution respectively. He incubated these emulsions, made bacterial counts at intervals up to one hundred and twenty hours and found that there is a marked diminution in the number of bacilli contained in the tuberculous gland emulsions as compared with the other two fluids. We have not had the opportunity to repeat this work.

REFERENCES.

1. Livierato. Riforma Medica, xxv, 286: cit. Zeitsch. f. Immunitätsforsch. Teil. II., Ref., 1909, i, No. 3, 235.
2. Arloing, S. Le Progrès médical, 1886, No. 6, 291.
3. Moore. Journal of Pathology and Bacteriology, vi, 98.
4. Baumgarten, P. Baumgarten's Jahresbericht, 1899, footnote, 459.
5. Pizzini. Zeitschrift fur klin. Med , 1892, xxi, 329.
6. Weichselbaum and Bartel. Wien. k Woch., 1905, No. 10.
7. Kälble. Münch. med. Woch., 1899, No. 46, 622.
8. Macfayden and MacConkey. British Med. Journal, July 18, 1903, No. 1, 123.
9. Harbitz. Journal of Infect. Dis., 1905, ii, 143.
10. Perez. Cent. f. Bakt. etc., Ref., 1898, xxiii, 404.
11. Manfredi. Virchow's Archiv., 1899, clv, 335.
12. Manfredi and Frisco. Cent. f. Bakt., etc., Ref., 1903, xxxii, 295.
13. Bartel, J. Wien. klin. Woch., 1905, No. 34.
14. Bartel, J. Wien. klin. Woch., 1905.
15. Bartel and Neumann. Cent. f. Bakt., etc., Orig., 1906, xl, 518.
16. Newmann and Wittgenstein. Wien. klin. Woch., 1906, No. 28, 858.
17. Bartel and Neumann. Wien. klin. Woch., 1907, Nos. 43 and 44.
18. Bartel and Neumann. Cent. f. Bakt., 1909, Abt. 1, xlviii.
19. Bartel. Wien. klin. Woch., 1909, No. 4, 117.
20. Rodet. Compt. Rend. Soc. de Biol., 1903, lv, 1109.
21. Rodet. Compt. Rend. Soc. de Biol., 1903, lv, 1111.
22. Livierato. Loc. cit.
23. Fontes. Centr. f. Bakt., etc., 1909, Orig., l, No. 1, 78.

III. FURTHER NOTES ON DIFFERENCES IN PRECIPITINS PRODUCED BY TUBERCLE BACILLI.*

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In some studies published in this journal (Vol. XII., 1904, page 242), certain differences were described between precipitins produced by injections of filtered extracts of tubercle bacilli and those produced by the washed residues after thorough extraction with .5 per cent $NaHCO_3$.

It was found that sera from the injected hares differed quantitatively and qualitatively when tested with the extracts or emulsions of residue respectively. The inference was drawn that sufficient difference existed between the proteins of the tubercle bacilli to account for their different behavior as precipitinogens, but the question was not quite settled. Experiments were continued with interruptions during the following two years with somewhat indifferent results, and have therefore remained unpublished hitherto. Since the matter has a practical bearing in connection with tuberculin therapy and especially the relative action of tuberculin solutions or extracts and the T.R. or residue, it seems worth while to record these further observations.

In the intervening years the literature of this subject contained but little that related directly to tuberculosis, although the general subject of precipitins and agglutinins has been exhaustively studied.

The quantitative relations in the agglutination of tubercle bacilli were examined by Karwacki and Benni[1] with the result that they found that the agglutinable substance was analogous to that of cholera and typhoid in binding agglutinin. It possessed the power to bind much more agglutinin than was needed to produce agglutination. The highest titer obtained in the sera of three rabbits treated with tubercle bacilli was one thousand five hundred units (= 1 to 1,500

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parts tubercle bacilli emulsion). The more concentrated the emulsion employed in testing sera, the lower the titer.

By the use of sera from diseased individuals Bonomé [2] has claimed to differentiate between human and bovine tubercle by means of a precipitin test. Extracts of human and bovine tuberculous tissue were precipitated by the homologous serum. This has not been confirmed in the hands of others (Dammann and Stedefelder,[3] Zwick [4]). Clinical application of the precipitin test has also been attempted with indifferent success in tuberculosis by Szaboky,[5] Stoerk,[6] and Porter.[7] So many apparently normal persons give positive results, as in the agglutination test, that little specific value attaches to it. Porter has made the most thorough study on six hundred and eighty-two human sera, carefully controlled under a variety of conditions with the presence and absence of salts, phenol, etc. Of the three hundred and eighty-one sera from tuberculous persons, twenty-five were from patients under tuberculin treatment. The author noted no larger percentage of reactions but some increase in its intensity in the latter. Both Szaboky and Porter made comparisons between the precipitin reaction and the complement deviation test, and Porter found, especially in advanced cases of tuberculosis, that the sera often deviated complement in the presence of bacillary extract but produced no trace of precipitate.

Our experiments were all made with the sera of Belgian hares or rabbits treated with either bacillary extract or residues. The apparent differences in the precipitins did not a priori seem to be due to fundamental differences in the protein constituents of tubercle bacilli, and therefore other explanations were sought for by a more detailed study of the relation of the reaction to modifying influences such as time, in absorption tests, greater or less alkalinity, etc.

We also tried to increase the precipitin strength of the sera by longer treatment of the animals and the use of larger doses of antigen. Thereby we should expect to obtain more sharply defined differences if they actually were distinct. In this we were disappointed and high readings were

not attained, — in fact some animals produced scarcely any precipitin. An immunized cow was also injected with bacillary filtrate with even less success in producing a visible precipitin reaction, though complement deviation tests revealed the presence of antibody.

The highest titer for filtered bacillary extract reached by hares immunized with the same was 1 : 33, while sera from hares immunized with bacillary residue agglutinated suspensions of the fourth extract (see Appendix) as high as 1 : 750.

The average was, however, less than 1 : 400 for Koch's agglutination emulsion (D.m. Woch., 1901).

Different concentrations of extract and emulsions were tried in testing sera to increase the delicacy of the reaction, and we found, contrary to our first observations, that filtered extracts could be precipitated by residue sera as high as 1 : 50.

The question whether or not agglutinins and precipitins are identical antibodies has a possible bearing on our observations as it has not been satisfactorily shown that they are necessarily different. Wasserman[8] has showed that greater amounts of precipitating substance are necessary for precipitation than for agglutination. This source of fallacy must be considered in the comparisons between our sera; and were it not that our strongest extract sera were weak in agglutinating power and vice versa, we should attribute any difference to the above fact. On the other hand, our further tests in absorbing the agglutinin or precipitin, respectively, lead to the conclusion that all the active substances of this kind can be removed by treating the sera with an excess of extracted tubercle bacilli. The same result follows prolonged incubation with a moderate amount of bacillary residue as previously determined.[9] The following examples are illustrative of complete and partial absorption of the precipitin:

BACILLARY (FILTERED) EXTRACT PRECIPITIN.

Experiment 223 B:

Rabbit No. 10, weight 1,830 grams. Immunized intraperitoneally from Jan. 25 to Aug. 8, 1905, with filtered extract R1 tubercle bacilli $NaHCO_3$ .5 per cent. Bled to death Aug. 16, 1905.

Precipitin titer, 1 : 15 bacillary extract (incubated twenty-four hours).

Agglutinin titer, 1 : 20 Koch agglutination emulsion (incubated twenty-four hours).

Aug. 22, 1905: 2.5 cubic centimeters serum No. 10 + excess of bacillary residue; incubated two hours; centrifugalized, pipetted, and filtered. Tests on extract and emulsion showed complete loss of both precipitin and agglutinin activity.

Rabbit No. 5, weight 2,000 grams. Immunized intraperitoneally from Aug. 24, 1904, to July 8, 1905, as No. 10. Bled to death July 18, 1905.

Precipitin titer, 1 : 33.

Agglutinin titer, 1 : 50.

July 20, 1905: 3 cubic centimeters serum No. 5 + excess of bacillary residue. Incubated three hours; centrifugalized and pipetted. Tests showed complete loss of agglutinin and partial loss of precipitation power, reducing the titer to 1 : 10 for bacillary extract.

The result in the second rabbit indicates how a strong precipitin serum may retain activity when the affinity for the antigen is feeble yet positive enough to bind some of the precipitin. It is even possible that any inert substance in emulsion may have absorbed some of the precipitin. This point was not controlled.

In view of the low activity of sera obtained from filtered extracts, we employed the residue for most of the animals used in further work.

Bacillary residue precipitin. — At the outset we had not noted any precipitin for the filtered extract in sera immunized with residue. This was found, however, in further tests, and the fact does away with the theory of any radical difference between our precipitins. Nevertheless a comparison of the titer respectively of the extract and residue sera brings out a certain suggestion of difference.

No.	Filtered Extract : Serum Dilution.	Emulsion of Residue : Serum Dilution.
Exp. 211. No. 5 hare	1 : 2 (?)	1 : 25
" " " 6 "	0	1 : 200–300
" 212. " 6 "	0	1 : 200 +
" " " 3 "	0	1 : 100
" " " 1 "	0	1 : 50
" " " 2 "	0	1 : 100
" " " 5 "	1 : 20 +	1 : 133–200
" " " 7 "	1 : 5	1 : 100
" 222B. " 2 rabbit.....	1 : 33	1 : 200
" " " 3 "	1 : 10	1 : 50
" 223B. " 4 hare	1 : 20–50 (?)	1 : 200–400 (?)
" " " 7 rabbit.....	1 : 15	1 : 100
" " " 4x "	1 : 15	1 : 20
" 225. " 3 hare	1 : 15 +	Not titrated.
" " " 4 "	1 : 20 +	1 : 50
" " " 5 "	1 : 15	Not titrated.
" 233. " 2 "	1 : 50–75 (?)	1 : 250
" 234. " 3 "	0	1 : 100

At first sight the irregularities bear no relation one to another, yet it can be seen that save for the first six sera there is a fair correspondence between the precipitin strength against the extract and residue respectively. In explanation of these differences there are several points to be mentioned.

The first is that we found the stronger extracts with $NaHCO_3$ were more easily precipitated; *i.e.*, those richer in protein. Hence we found the first extracts more readily precipitated than the second, and so on. Furthermore, we found that the emulsions to which NaCl was added to make .85 per cent in addition to the .5 per cent $NaHCO_3$ already

present gave higher titrations.* Then we found that by neutralizing the emulsion of residue with $\frac{N}{10}H_2SO_4$, higher readings were obtained though not with the filtrate, as a rule. A N/NaCl extract was frequently employed for comparison. The restraining influence of an alkaline reaction may, however, have been a factor in our earlier experiments with weak extracts and thus prevented precipitate formation.

Finally, the residues used for immunizing the animals probably varied in the amount and absorbability of the bacillary protein contained therein, since the bacilli were not pulverized as in the preparation of T.R. tuberculin. Hence only gradations of difference should have been expected in the precipitins resulting from these preparations.

Extracts and residues of two cultures, R1 and H33, were used in this study without developing notable differences in precipitability or antigen properties. Such differences as did show in the precipitin titer may be accounted for by the varying protein content of the preparations used.

A brief mention only need be made of the absorption experiments with residue sera, since the same results were obtained as with the extract sera. Fourteen different sera were treated with bacillary residue from one to twenty-four hours. Seven were completely inactive after one treatment, for both extract and residue. No. 4, 223B, was made inactive only after a second treatment with residue; or the same result was accomplished by a second treatment with extract four or five hours after removal of the first and second precipitates.

Incidentally, some sera were tested with the emulsion of homogeneous bacilli after the method of Arloing and Courmont. No agglutination was noted above 1:10 in one serum that gave 1:50 with Koch's emulsion. The latter was of course more nearly homologous to the antigen used on the animals. The results may be summed up as follows:

1. The apparent differences between precipitins produced

* On the other hand, in the table are included only titrations with either $NaHCO_3$ emulsions of residue or Koch's bacillary emulsion in N/NaCl.

by filtered extracts of tubercle bacilli and the extracted residues are not fundamental.

2. By prolonged or repeated treatment of the sera with the residue or extract the precipitins for both can be absorbed.

3. Quantitative differences in the antigen or precipitinogen and its absorbability by the animals explains in great part the difference in the resulting sera, as well as the physical and chemical conditions present in the tests in vitro.

REFERENCES.

1. Karwacki (L.) and Benni, W. Cent. f. Bakt. Orig., 1906, xlii, 252.
2. Bonomé. Cent. f. Bakt. Orig., 1907, xliii, 391.
3. Dammann and Stedefelder. (Ref.) Folia Serologica, 1909, iii, 172.
4. Zwick. (Ref.) Folia Serologica, 1909, iii, 173.
5. Szaboky. Zeits. f. Tuberk., 1909, xiv, 276.
6. Stoerk. Wien. kl. Woch., 1909, lix, 417.
7. Porter, A. E. Journ. Infect. Diseases, 1910, vii, 87.
8. Wasserman. Handbuch d. Immunitätsforsch., Kraus and Levaditi, ii, 669.
9. Loc. cit., Journ. Med. Res., xii.

(IV.) A CONTRIBUTION TO THE QUESTION OF CATTLE IMMUNIZATION AND THE TRANSFORMATION OF THE HUMAN INTO THE BOVINE TYPE OF TUBERCLE BACILLUS.*

E. R. Baldwin, M.D.

The observations here recorded have a two-fold interest. First, because they illustrate the impracticability and danger of attempts to immunize cattle with virulent tubercle bacilli of human origin, and second, the apparent persistence of the human type of Bacillus tuberculosis after a prolonged residence and multiplication in the tissues of a bovine subject.

The facts relate to an occurrence in the course of some tentative immunization experiments on two cows by the so-called Koch-Schütz method with the object of obtaining milk which should contain anti-bodies. A fresh culture of human origin and type (H38) had been isolated for this purpose shortly before the bovine inoculations. The definitely human type possessed by this culture was evinced in the following observations:

Cultures and inoculations of B. tuberculosis "H38." — The original culture of H38 (see Appendix) was made directly from the sputum Aug. 28, 1905, upon Hesse's agar. The source was an acute pneumonic tuberculosis in a female aet. thirty-seven. The disease was fatal nine months after its onset. Transfers from the agar plate smears on Aug. 28, 1905, were made as follows:

First transfer Sept. 2, 1905, to Loeffler's beef serum; second transfer Sept. 15, 1905, to ibid.; third transfer Sept. 30, 1905, to ibid.; fourth transfer Oct. 15, 1905, to glycerine broth, which latter was used for the inoculations; *i.e.*, two months after isolation from the sputum. The morphology of this bacillus as found in the original sputum was that of the characteristic human type. It comprised long, slightly

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curved rods with more or less beaded forms. The growth was fairly rapid and abundant on all media.

Inoculations. — At the same time that the cows were inoculated for immunization five guinea-pigs were infected with the same emulsion of H38 tubercle bacillus. Three were inoculated subcutaneously in the right groin with one cubic centimeter (= .00025 gram) each. Two were inoculated intraperitoneally with .5 cubic centimeter (= .000125 gram) each. The virulence was normal for human tubercle bacilli as may be seen by the time of death.

No.	Weight 30-x-5.	Inoculation.	Result.	Post-mortem.
1...	430	Subcutaneous.	† 63d day.	Extensive general caseous tuberculosis.
2...	490	"	† 45th "	Extensive general caseous tuberculosis.
3...	510	"	Killed 67th day.	General tuberculosis; little caseation.
4...	400	Peritoneal.	† 13th day.	General caseous tuberculosis.
5...	520	"	† 47th "	General caseous tuberculosis; culture recovered from spleen and lung.

Inoculations of rabbits were not made with the original culture but only after two passages through guinea-pigs and recovery in pure culture, as "$H38_{**}$" eight months later.

June 15, 1906: Two rabbits were inoculated as follows:

No. 1, gray hare, weight 1,600 grams; No. 2, white rabbit, weight 1,630 grams.

Each received intravenously 3.25 cubic centimeters N/NaCl emulsion of a fresh culture of $H38_{**}$ on Loeffler beef serum from June 1, 1906. The emulsion was made up .001 gram to 1 cubic centimeter and then centrifugalized ten minutes to remove clumps. Only the supernatant emulsion was inoculated, which represented an unknown but very small amount of the original weight of tubercle bacilli.

No. 1 died July 7, 1906 (twenty-three days). Very much emaciated. A few scattered gray tubercles were found in each lung. The spleen was enlarged (tubercle bacilli in smears, one to two fields). The kidneys revealed a few foci of beginning tuberculosis.

No. 2 killed March 12, 1907 (nine months). In excellent condition, weight 2,265 grams. No signs of tubercle in the lungs. Some old cicatricized tubercles in the kidneys; otherwise normal.

Result. — The result of these inoculations did not favor the idea of an unusual virulence for this culture on guinea-pigs or rabbits.

Comparison with bovine type. — The contrast in virulence for rabbits between H38$_{**}$ and a true bovine stock isolated in 1902 known as "B1" was very striking. At the same time that the above inoculations were made, two rabbits were inoculated with an emulsion made up in the same way and centrifugalized simultaneously, but with one-half the weight of B1 culture from Loeffler beef serum grown since June 2, 1906.

No. 3, white rabbit, weight 2,100 grams; No. 4, gray hare, weight 2,310 grams.

No. 3 died July 2 (eighteen days). Miliary tuberculosis in both lungs, especially the right inferior caudal lobe; minute tubercles in left lung; spleen and liver swollen; probably abundant miliary tubercles.

No. 4 died July 27 (forty-three days). Emaciated; extensive caseous tuberculosis in both lungs; foci in kidneys.

It is known that intravenous rabbit inoculations are not always reliable for determinations of the virulence of bacillus tuberculosis, yet when the amounts used are not excessive and are freed from large clumps of bacilli by the centrifuge, they have in our hands proved the sharp contrast between the human and bovine stocks.

Bovine Inoculation.

Oct. 23, 1905: No. 14, yearling heifer of Holstein stock; well nourished; weight 425 pounds; No 15, two-year-old cow (Holstein); seven months pregnant; weight 650 pounds.

October 24: Both have normal temperature.

Oct. 25, 1905, 9.30 A.M.: Tuberculin test. — No. 14 received .350 cubic

centimeter No. 130 O.T.; No. 15 received .500 cubic centimeter No. 130 O.T. Highest temperature taken two-hourly was: at 7.30 P.M., 102° F. for No. 14; no reaction; 102.5° F. for No. 15.

October 26: Highest temperature 5 P.M., 101.8° F. for No. 14; 101.6° F. for No. 15.

October 30: Both animals were inoculated in the right jugular vein with .020 gram moist-weight H38 tubercle bacilli from broth culture grown fifteen days. The bacilli were emulsified in N/NaCl .002 gram to 1 cubic centimeter. Each animal received 10 cubic centimeters successfully at 1 P M. Highest two-hourly temperature 7 P.M., 102.2° F. for No. 14; 102 4° F. for No. 15.

October 31: Highest two-hourly temperature 9 A.M., 104° F. for No. 14; 5 P.M., 102° F. for No. 15.

History of Cow No. 14. — The temperature of No. 14 subsided to normal on the third day and remained so until November 4 when she was removed to a farm for a prolonged stay. Unfortunately no further record was made of the temperature until eighteen months afterwards. She lost ten pounds after inoculation but later regained weight and developed normally. She remained in excellent condition for one year and a half, meantime bearing a calf which was born about April 10, 1907. The calf was suckled until May 21 when it died following an injury from poking by other cattle. An adherent pericardium was the only abnormality found and this was clearly due to recent trauma.

On May 31, 1907, the cow was tested with tuberculin along with others at the farm by a State veterinarian and gave a definite reaction (105.5°). She was then in poor condition and on being returned to the laboratory stable it was discovered that she had a swelling on the inner aspect of the left knee about the size of an orange. This was hard to the touch and had the appearance of a tuberculous cold abscess, from which the animal suffered little inconvenience.

Some of the milk was obtained on May 29 and inoculated into two young guinea-pigs intraperitoneally. They were killed on July 18 (fifty-one days) and nothing abnormal found.

From June 12th to the 17th the temperature was taken and ranged from 102.4° to 102.8° F. It was then decided

to slaughter the cow immediately after another tuberculin test, for the purpose of revealing concealed disease more distinctly as a result of the focal reaction induced.

June 17, 1907. Tuberculin test at 8 P.M., .500 cubic centimeter No. 140 O.T.

June 18, 1907. Highest temperature 6 A.M., 104.5° F.

The animal was slaughtered at 9 A.M. by Dr. W. H. Kelly, Chief New York State Veterinarian, who kindly made the post-mortem examination on the same day.

Autopsy. — General appearance poor; hair rough and pelvis prominent.

Abscess: A tumor the size of an orange was situated on the inner aspect of the left hind leg, at the tibio-tarsal joint. It involved the deep fascia outside the capsule, and had thick vascularized walls, somewhat congested (tuberculin reaction?). A small synovial cyst was found on the outer aspect of the same joint which had no communication with the abscess. The latter when opened aseptically was found to contain semi-fluid inodorous pus. Cultures made directly from the abscess on Loeffler beef serum produced a good, uncontaminated growth of tubercle bacilli on two tubes by July 10 (twenty-two days). They were designated "H38 B." The pus contained numerous tubercle bacilli in every smear. No pyogenic organisms were grown from the abscess. The regional lymph nodes in the groin were not noticeably enlarged, nor was there any serious interference with the function of the joint from the presence of the abscess.

Lymph glands: Both inguinal, posterior, gluteal, both mammary, both axillary, cervical, posterior auricular, all sub-maxillary, bronchial, lung hilus, mediastinal, mesenteric, hepatic, gastric, and lumbar glands were removed and incised in turn. All were of normal size and appearance except the lumbar, which contained two small nodules, neither caseous nor congested, although the gland as a whole was dark. Inoculations were made from a number of the glands and other tissues as hereafter noted.

The salivary glands, pancreas, and spleen were normal in appearance. The spleen contained a small supernumerary lobule at the extreme end. It was not caseous.

The mammary glands (functionating) were carefully cut into small pieces, and all parts appeared normal. Only one congested area was discovered in the sub-capsular portion. This was reserved for inoculation. The teats were normal.

Viscera: Liver, kidneys, uterus, stomach, and esophagus were normal. A small fibrinous clot was present in the pericardium; the heart appeared normal. No trace was found of the site of inoculation in the jugular vein or surrounding tissue.

Intestine: On the wall of the small intestine there were three small subperitoneal nodules, about five millimeters in diameter. They resembled normal lymph follicles when viewed from the intestinal lumen. On section one was found caseous, the others not. Smears and inoculations were negative to tubercle bacilli. The intestinal mucosa was normal throughout.

Lungs: The pleura of the left caudal lobe revealed an area of distinct roughness. Slight adhesions were also present over the superior aspect of the right caudal lobe. Some areas of peribronchial tissue near the pleural surface of the left caudal lobe inferiorly formed depressions which were darkly congested. These depressions from three to five centimeters in diameter were hard and evidently of tuberculous character when seen on section. Foci containing caseous and fatty substance in their centers were readily seen, varying in size from a millet seed to that of a pea.

Some isolated miliary tubercles of translucent appearance were seen situated peripherally to the main foci; also some calcareous nodules were noted in cutting into the lung tissue.

The congestion of the diseased lung was evidently caused by tuberculin administered twelve hours previous to death. The lungs were otherwise normal. Smears made from the cheesy tubercles were all negative as also was the result of inoculation into guinea-pigs of tubercles crushed in a mortar with N/NaCl. The inoculations were made on June

18, both subcutaneously in the right groin and intraperitoneally into young guinea-pigs as shown by the table:

TABLE.

No.	Weight.	Material.	Site of Inoculation.	Result.	Autopsy.
1.	290	Abscess pus.	Subcutaneous.	Killed 21st day.	Extensive caseous tuberculosis.*
2.	285	" "	Intraperitoneal.	Died " "	Extensive caseous tuberculosis.
3.	250	" "	"	" 20th "	Extensive caseous tuberculosis.
4.	230	Tubercle; left lung.	Subcutaneous.	Killed 30th "	Nothing abnormal.
6.	250	Tubercle; left lung.	"	" " "	" "
7.	230	Udder tissue.	"	" " "	" "
8.	210	" "	"	" " "	" "
9.	255	Udder lymph gland; left(?)	"	" " "	Enlarged and caseous inguinal glands; few tubercles in enlarged spleen.
10.	230	Udder lymph gland; left(?)	"	" " "	Enlarged and caseous inguinal glands; few tubercles in enlarged spleen.
11.	235	Left inguinal lymph gland.	"	" 22d "	Nothing abnormal.
12.	230	Left inguinal lymph gland.	"	" " "	" "
13.	220	Lumbar lymph gland.	"	" " "	" "
14.	170	Lumbar lymph gland.	"	Died 9th "	Enteritis; otherwise normal; smears from right inguinal gland negative to tubercle bacilli.
15.	235	Lung hilus lymph gland.	"	Killed 30th "	Nothing abnormal.
16.	265	Lung hilus lymph gland.	"	" " "	" "

*Cultures successfully recovered from lymph nodes and spleen.

Result. — The abscess contained virulent tubercle bacilli as did the udder lymph node on the same (?) side as the abscess, as shown by the guinea-pig inoculations. The other tissues were negative and the lung tubercles non-infectious, probably because long healed or because they contained too few living tubercle bacilli in the portions used. Microscopic

examinations have not yet been made. Cultures were successfully made from Guinea-pigs Nos. 1 and 2 on Loeffler beef serum, which were also designated "H38 B." Unfortunately lack of animals and facilities prevented inoculation of the pus into rabbits and calves at the time the material was fresh.

History of Cow No. 15. — After inoculation the highest temperature reached by Cow No. 15 was no greater than normal, and like No. 14 it remained normal until November 4. Thenceforth it began to rise and persisted during the succeeding nineteen days. The highest point (105°) was reached on November 10, eleven days after the inoculation. During the following week it subsided to normal (between 101 and 102°), and remained so until Jan. 1, 1906, when the record was discontinued.

The calf was born Jan. 9, 1906, and immediately slaughtered. Nothing abnormal was found. The cow's milk was used for agglutination tests without positive results, the whey having been employed in various ways. The milk was also supplied to two hospital patients with progressing disease, without noticeable influence on their temperature or other symptoms. No inoculation experiments were made with this cow's milk, as far as our records show.

The cow maintained a fair weight and condition during lactation and when taken to pasture, May 27, 1906, weighed five hundred and forty pounds. Oct. 15, 1906: On return she was found in excellent flesh and condition. October 22: Tuberculin test, .500 cubic centimeter No. 130 O.T. Reacted to 106° in ten hours. Subsequently she was injected during four months repeatedly with filtered water extract of tubercle bacilli, both subcutaneously and intravenously, with the intention of producing specific precipitin in her blood. This was unsuccessful so far as the production of visible precipitates was concerned. Even the agglutination titer reached only 1 : 100, whereas the serum before inoculation had been 1 : 75 with Koch's emulsion. No fever reactions occurred during the injection of bacillary extract, which was

evidence of relatively weak dosage, twenty-five cubic centimeters being the largest amount injected at one time.

During the summer of 1907 the cow was in fine condition and being returned from pasture was again tested with tuberculin. Oct. 25, 1907: Conjunctival tuberculin test, .050 cubic centimeter of two per cent 143 T.P. instilled; no reaction. October 26: No reaction. November 5: .500 cubic centimeter 140 O.T. injected; no reaction. November 6: No reaction.

Autopsy. — The animal was slaughtered early in December, but a careful autopsy was not possible on the day of death. Only the trachea and lungs were carefully examined and these on the following day. So far as superficial gross appearances could give evidence, there was nothing abnormal in the viscera. The glands of the lung hilus on incision revealed a white cicatrix in the largest of the group. The lungs appeared normal on section.

No inoculations, cultures, or microscopic examinations were made, but there was good reason to think that the infection was completely overcome and healed in this cow.

Cultures and inoculations of the "H38 B." — After recovery of the culture from the Cow No. 14, June 18, 1907, no difference could be observed in the morphology as compared with the original culture. The rods obtained directly from the pus were also long and characteristic of the human type. Many degenerated and poorly stained rods were present, but the combined fuchsin-Gram staining method of Much was not known to us at the time, and therefore not used to detect granular forms. After passage through the guinea-pigs the culture recovered from lymph nodes and spleen showed no different morphology.

Inoculation of rabbits. —The cultures used for rabbit inoculations were isolated directly from the abscess pus and also from the guinea-pigs, which were previously inoculated with the pus. They were well grown Loeffler beef serum

cultures planted eighteen days before and mixed in an emulsion containing .002 gram to 1.5 cubic centimeters N/NaCl. They were not centrifugalized. Six hares were each inoculated intravenously with 1.5 cubic centimeters (= .002) on July 29, 1907.

The table presents the result:

TABLE.

No.	Weight.	Result.	Autopsy.
1.	1450	Died 2d day.	Coccidiosis of liver.
2.	1100	" 23d "	Spleen swollen; tubercle bacilli positive in smears; otherwise normal.
3.	1390	Killed after 5 months.	Well nourished; cold abscess on left hind knee joint. Lymph glands on all extremities caseated. Lung showed one circumscribed caseous nodule. Kidney also contained one nodule.
4.	1520	Died 7th day.	Emaciated; coccidiosis. No macroscopic tubercle.
5.	1570	" 11th "	Emaciated. Nothing abnormal in macroscopic appearances.
6.	1400	In good condition 6 months later. (Used in another experiment Jan. 10, 1908.)	

Result.—Three animals (Nos. 1, 4, and 5) must be excluded because of their early death and complications, but the other three are available as evidence against any increased virulence as compared with the original culture.

Clearly there was no increase of virulence for hares by passage of this culture through the cow and guinea-pigs considering the large dose of tubercle bacilli inoculated. It was a misfortune that we had no facilities at the time to inoculate calves, but enough was shown by its low virulence for rabbits to indicate what might be expected. There was nothing to indicate a transformation into a bovine type by the nineteen months' sojourn of this bacillus in the cow. Yet the numerous clumps of bacilli found in the abscess pus clearly showed that they had multiplied and were not simply encysted masses lodged there as an embolus at the time of inoculation, although the focus most likely originated in that way. The localized disease in the lung was quite overcome,

but probably trauma or use of the leg gave the impetus to the abscess formation.

A further confirmation of the essentially human origin and persistence of type of this culture is found in the observations of Prof. Theobald Smith, who kindly estimated its acid production in broth culture. This test was unfortunately delayed unavoidably for two and a half years, but the subcultures thus tested have the property of acid reaction production which is a distinguishing trait of the typical human type, as found by Professor Smith.

Our experience with this culture corresponds closely with the results obtained by Weber [1] with a human culture passed through four calves in succession, which retained its characteristic human type after six hundred and eighty-five days' residence in the bovine species. This was demonstrated by cultures directly from a gland and by rabbit experiments. Moreover, the second passage culture inoculated into Cow 159 [2] in a dose of .050 gram produced an effect almost identical to that of our own H38 (which, by a coincidence, had the same number as that of Weber). Not only did fever result for some weeks, but after eight and a half months localized foci of tuberculous origin were found in synovial bursæ of both carpal joints of the forelegs, from which guinea-pigs were infected. Our case differs, however, from any of Weber's more extensive experiments in one particular, namely, that the culture retained vitality and type for nineteen months in the same animal, whereas the experiment quoted, as well as those by Weber and Titze,[3] also Weber,[4] Schütz, Titze and Holland, published later on the persistence of human type bacilli in the bovine tissues, appeared to show that they died out after six months. Schroeder and Cotton [5] had already showed that living human bacilli could be demonstrated after repeated intravenous inoculation of tubercle bacilli after ten months, by guinea-pig inoculation of lung tubercle. They also demonstrated them as long as one and a half years in a localized abscess at the site of a subcutaneous inoculation. Our experiments have a further interest in view of the recently published work of Eber,[6] who produced

transitional types of virulence for calves and rabbits from two out of seven cases of pulmonary tuberculosis in man. Eber states that when certain sputum bacilli are passed through guinea-pigs and the splenic tissue or culture bacilli from the guinea-pig are inoculated intraperitoneally into young calves a fairly mild infection results. If, however, the calf tubercle is passed through guinea-pigs into a second calf an acute miliary tuberculosis may result.

Our experience with one culture, so far as it bears on the question of increased virulence through passage into guinea-pigs, is diametrically opposite; and in respect to the time during which the bacillus was sojourning in the bovine tissues, the opportunity was much greater for the development of a transitional type. We are aware that the lack of further inoculations into calves weakens the value of the evidence, but the rabbit inoculations go far to show the persistence of type for this culture at least.

CONCLUSIONS.

1. Virulent cultures grown directly from human sputum may cause active infection in the bovine species when inoculated intravenously.

2. The disease is generally localized and becomes healed, but is a sufficient danger to preclude such methods of immunization for cattle.

3. The disease may remain latent in the cow and later become active in a local focus, possibly owing to parturition, lactation, or trauma.

4. A culture of typical human type when sojourning and presumably multiplying in the bovine tissues for nineteen months may retain its original morphology and virulence for guinea-pigs and rabbits, yet not necessarily exhibit an increased virulence. It may also retain the ability of the human type to produce an acid reaction in broth cultures.

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REFERENCES.

1. Weber, H. Tuberkulose-Arbeit. a. d. Kaiserlich. Gesund., 1907, No. 6, 78–87.
2. Loc. cit., 79.
3. Loc. cit., No. 7, 10.
4. Loc. cit., 1908, No. 9, 27–50.
5. Schroeder, E. C., and Cotton, W. E. U.S. Bureau An. Indust. 1905, Bull. No. 52, part III.
6. Eber, A. Münch. med. Woch., 1910, No. 3, 115.

A REPORT ON THE USE OF "ANTIFORMIN" FOR THE DETECTION OF TUBERCLE BACILLI IN SPUTUM, ETC.*

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Antiformin is the patented name of a disinfectant which was introduced in 1900 by Victor Tornell and Axel Sjoo of Stockholm, as a cleansing material for fermenting vats in breweries, but it is only since the investigations of Uhlenhuth and Xylander in the Kaiserliche Gesundheitsamt in Berlin in 1908 that it has come into prominence in bacteriological and hygienic work. Its composition is simple: namely, equal parts of liquor sodæ chlorinatæ (B.P.),† and a fifteen per cent solution of caustic soda. Its activity seems to be due to an intense oxydation. It has a strong germicidal action in weak solutions (two to five per cent) on the ordinary cocci and bacilli — staphylococci, streptococci, pneumococci, typhoid, colon, etc. — killing them rapidly, five minutes at most being sufficient. In this respect "antiformin" acts more rapidly and surely than either of its component parts used alone. It is an almost colorless liquid with a strong odor of chlorine and is strongly alkaline. It keeps well without particular precautions being taken, that used by us after keeping for two months being apparently as effective as when fresh. Its efficiency as a germicide is in part due to its ability to dissolve and to render homogeneous the various substances in which the bacteria are found: sputum, feces, pus, urinary sediment, and even small pieces of organs; thus enabling the fluid to reach the bacteria in the interior of cells and masses of tissue which are coagulated on the outside by

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* Studies from the Saranac Laboratory, March 8, 1910.

† Sodium carbonate 600
Chlorinated lime 400
Aqua dest. 4,000

Dissolve the sodium carbonate in 1,000 cubic centimeters of the distilled water. Triturate thoroughly the chlorinated lime in the remainder of the water. Filter. Mix the two and filter again.

so many of the commonly used disinfectants, and thus prevent the penetration of the fluid used. An interesting fact was noted during Uhlenhuth's investigations, namely that the acid-fast group of bacilli — tubercle, smegma, timothy, butter, etc. — were extremely refractory to the action of antiformin and that only upon using concentrated solutions for a long time were these bacilli killed. This was proved in the case of tubercle bacilli by guinea-pig inoculation. The theory suggested and the one that seems most plausible is that this resistance is due to the biochemical composition of these bacilli, the fatty capsule which is present and which gives to them their acid-fast property, acting as an impervious coating which resists the dissolving action of the antiformin and so protects the protoplasm of the bacilli from its germicidal action. This fact has opened up a field of usefulness for antiformin in the examination of sputum. The tenacious thick masses so often found in sputum and which contain tubercle bacilli in their interior are broken up and dissolved by the antiformin while the bacilli are not affected either as regards their morphology or their viability; thus enabling us to mass the bacilli by sedimentation or to grow them direct from the sputum after the contaminating organisms have been killed. The method commends itself by its simplicity and with a little practice yields very constant and satisfactory results.

Our procedure with this preliminary study at the Saranac Laboratory was to add to ten cubic centimeters of sputum 2.5 cubic centimeters antiformin, thus giving a strength of twenty per cent antiformin. Where the sputum was very thick and tenacious or not in sufficient quantity, a smaller amount was diluted to ten cubic centimeters with distilled water, thus giving a constant strength of twenty per cent antiformin. The tubes used were ordinary centrifuge glasses which had been cleansed and kept in a mixture of potassium bichromate and sulphuric acid* to eliminate the possibility

* Rub up some potassium bichromate with sulphuric acid for two minutes, allowing the acid to take up as much of the bichromate as it will. Pour off the acid and repeat the process.

of tubercle bacilli from other sources being present. Just prior to use the tubes were washed thoroughly with distilled water. The tubes when filled with the antiformin-sputum mixture were corked with unused corks, well shaken and allowed to stand for twenty-four hours at room temperature. If it is desired to make cultures or to use the material for inoculation it should be kept in the dark, as even diffuse light will attenuate the bacilli and so introduce a source of error into the investigation. After twenty-four hours the tube is again well shaken and then thoroughly centrifugalized. The supernatant fluid is now drawn off, the tube filled with sterilized normal sodium chloride solution, again corked, shaken and centrifugalized. The washing is done similarly a second time to rid the sediment of all alkali. After centrifugalizing a third time, the fluid is drawn off and the sediment smeared on a slide with a platinum loop or capillary glass tube, dried, fixed and stained in the ordinary way, or transferred to culture tubes and incubated. If the sediment has been thoroughly washed it will adhere to the slide perfectly, although some investigators have recommended using an albumen-glycerine fixative or smearing the slide with some of the untreated sputum from which the sediment is derived. Different investigators have used varying strengths of antiformin, from five to fifty per cent, and have left the mixture for varying lengths of time, but twenty per cent acting for twenty-four hours at room temperature, or four to six hours at 37°, gives excellent results. Some allow the sediment to settle down in sedimenting glasses, but this takes longer and is less certain to obtain all the bacilli present. Another variation of the method is to use the sediment without washing; fixing it to the slide as above mentioned. Neutralization of the mixture with a weak acid has been tried but is not recommended, as the separation of the bacilli is interfered with. Some add a little ninety-six per cent alcohol to the mixture before centrifugalizing to lower the specific gravity and aid the sedimentation. Hüne adds absolute alcohol, then a few drops of acetic acid, then distilled water and ether, centrifugalizing at different steps in

the process, his object being to render sedimentation more certain. In the majority of cases, however, the simpler proceeding, detailed above, will suffice and commends itself by its simplicity to those who have not laboratory facilities for following out elaborate processes.

On adding the antiformin to the sputum, this is thinned and liquefied almost immediately, but it is advantageous to leave it longer in order that the solution may proceed further and that all the sticky masses may be broken up. The sediment when examined is found to consist of débris, swollen and distorted cells, epithelial and leucocytic; adventitious substances in the sputum, as dust and dirt, sometimes swollen and faintly staining bacteria, and tubercle bacilli, if present. These latter are unaffected by the antiformin and retain their shape and staining properties perfectly if thoroughly washed. This is a distinct improvement on some of the other methods of digestion which distort the bacilli and render staining unsatisfactory and indistinct. It is not necessary to rid the sediment entirely of all elements except bacilli, for the swollen cells, etc., give a background for the bacilli which makes the search less fatiguing for the eye.

Cultivation of the bacilli direct from the sputum after treatment with antiformin was tried in a few cases, but in only two were we successful. Failure may have been due to using too concentrated antiformin or to leaving the tubes in the light, as was done at first. The sediment, after thorough washing, was transferred freely to serum tubes and in from seven to ten days colonies were apparent. The antiformin does not seem to interfere with the viability of the bacillus, as the growth occurs at the same rate as in the case of untreated bacilli. On some tubes the tubercle bacilli were quite pure, in others there would appear one or two colonies of slowly growing cocci or in one case a mould from which the tubercle bacilli were easily separated. Plates of Hesse's and plain agar were tried but without success. No animal experiments have as yet been tried by us but the advantages of this method are manifest: namely, the securing of a specimen free from contaminating organisms and in which the tubercle bacilli, if present, are concentrated in a

small amount of material. Other observers have examined various other substances for tubercle bacilli and have claimed success in pus from cold abscesses, feces, urinary sediment, and blood. Seeman finds the method well adapted for examining feces in cases of tubercular enteritis and in those where no sputum is available, but the suspicion exists that the sputum is being swallowed, as in children with ulcerative pulmonary tuberculosis. He also claims to have discovered bacilli in fifty per cent of cases of tubercular abscess in which the pus was examined. He has found tubercle bacilli in the heart blood in two cases of phthisis, which came to autopsy, and suggests that by drawing the blood from a vein it might be a means of differentiating miliary tuberculosis from typhoid or sepsis. Lagrèze has found tubercle bacilli in glands which did not appear caseous macroscopically.

Our experience with antiformin-treated organs is limited. The organs to be examined were cut into small pieces and shaken up in distilled water; antiformin, twenty per cent, was added and then the mixture was incubated for twenty-four hours at 37° C., after which time the organs were found almost completely macerated and ready for centrifugalization. The lungs, liver, and spleen of a tuberculous guinea-pig were examined in this way. The spleen showed a few bacilli, the other organs were negative. No bacilli could be found in smears directly from the organs. The lungs of a tuberculous cow were examined but maceration was not complete. Bacilli were, however, found in greater numbers than in direct smears.*

Our results with sputum were on the whole satisfactory and compare favorably with those of other observers. Before a specimen was declared to be negative it was examined by two observers independently, each making his own smears and doing his own staining. Eight cases with tubercle bacilli in smear preparation showed greatly increased numbers in five after treatment with antiformin, the same number approximately in two, and rather fewer in one.

* Dr. Brem, working in this laboratory in the summer of 1909, was able to isolate tubercle bacilli, by means of antiformin, in milk from a cow with a tuberculous udder. The method is also being successfully used in sputum examinations at the Adirondack Cottage Sanitarium.

These last three were among the first few examined and the result was due to a faulty and imperfect technic. Twenty-three cases with no bacilli in smears gave five positive results after antiformin treatment.

Cases.	T.B. present in Smears.	After Antiformin.	Cases.	T.B. not present in Smears.	After Antiformin.
1. Ci	+	++	1. S.P.	O	O
2. A.....	++	Fewer.	2. Fitz.	O	+
3. Ri	+	As before.	3. A.	O	O
4. M.M....	+	" "	4. In.	O	O
5. H.....	+	++	5. Lov.	O	O
6. Bi	+	+++	6. Ads.	O	O
7. Q.....	+	++	7. X.	O	O
8. H.....	+	++	8. Be.	O	+
			9. Sch.	O	+
			10. Por.	O	O
			11. Ja.	O	O
			12. Art.	O	+
			13. Xi.	O	O
			14. Sur.	O	+
			15. Kn.	O	O
			16. Dr.	O	O
			17. Pa.	O	O
			18. Be.	O	O
			19. Dr. O.	O	O
			20. Cr.	O	O
			21. Ca.	O	O
			22. Tr.	O	O
			23. Pa.–2.	O	O
Totals	8	8	.....	23	5

Meyer examined ninety-nine cases, negative in smears, and found fourteen positive after treatment with antiformin. Lagrèze, in fifty negative sputa, found bacilli in twenty per cent with antiformin. Rau, in eighteen negative smear specimens, found bacilli in five after treatment with antiformin and in every case in which bacilli were present in the smear they were found in greater numbers after such treatment. He also compared this method with the recently published ligroin method of Lange and Nitsche, and a combined antiformin-ligroin method, obtaining better results more easily with the simple antiformin than with the other more complex methods.

SUMMARY.

1. Antiformin provides a cheap, easy, and efficient method of examining sputum, in which tubercle bacilli are suspected or are few in number.

2. Solutions of antiformin which will kill ordinary contaminating organisms, leave tubercle bacilli intact, thus enabling us to cultivate the bacilli direct from sputum or to make inoculation experiments more certain, by eliminating the chances of sepsis.

3. Antiformin is useful for the examination of feces, urinary sediment, pus, and blood for tubercle bacilli.

LITERATURE.

Uhlenhuth and Xylander. B. klin. Woch., lxv, No. 29, 20th July, 1908.
Meyer. Tuberculosis, viii, No. 2, February, 1909.
Seeman. B. klin. Woch., lxvi, No. 14, 5th April, 1909.
Thilenius. B. klin. Woch., lxvi, No. 25, 21 June, 1909.
Hüne. D. med. Woch , xxxv, No. 41, 14 Oct., 1909.
Hammerl. Münch. med. Woch., lvi, No. 38, 21 Sept., 1909.
Rau. Hygien. Rundschau, xix, No. 23, 1 Dec., 1909.
Lagrèze. D. med. Woch., xxxvi, No. 2, 1910.
Lange u. Nitsche. D. med. Woch., xxxv, No. 10, 11 March, 1909.

APPENDIX.

DESCRIPTION OF CULTURES AND EXTRACTS.

A detailed history of the various cultures and other preparations employed in this work.

Cultures.

"R1." Bacillus tuberculosis hominis. Isolated in 1891 directly from a human lung, the patient having died of acute miliary tuberculosis. It was passed through one rabbit and has since been cultivated successively on glycerine-sheep serum, glycerine agar and glycerine broth. During the past fifteen years it has been grown upon broth containing three or five per cent glycerine and reacting slightly acid; a new transfer has been made nearly every month. Its virulence for rabbits and guinea-pigs was pronounced at first but at present the culture barely infects guinea-pigs, even in large doses, and then only very slight, localized or chronic disease results.

"H33." Isolated Sept. 18, 1903, directly from sputum on Hesse's agar, the source (Miss R. M.) being a case of acutely progressive tuberculosis. The virulence was pronounced for rabbits intravenously inoculated, and white rats were slightly infected.

"H34." Isolated Nov. 9, 1904, directly from sputum on Hesse's agar, the source (Mrs. D.) being a case of acutely progressive tuberculosis. The virulence was not determined for rabbits.

"H35." Isolated Nov. 13, 1904, directly from sputum on Hesse's agar, the source (Mr. H.) being a case of acutely progressive tuberculosis. The virulence was not tested.

"H36." Isolated November, 1904, from a guinea-pig inoculated with urine from a case of renal tuberculosis (Mrs. B.). The virulence was not tested.

"H37." Isolated Aug. 12, 1905, directly from sputum on Hesse's agar, the source (Dr. E.) being a case of chronic tuberculosis. The culture was of average virulence for guinea-pigs, but produced in rabbits only local nodules at site of inoculation subcutaneously, and fibrous foci in calves intravenously.

"H38." Isolated Aug. 28, 1905, directly from sputum on Hesse's agar, the source (Mrs. W.) being a case of acute pneumonic tuberculosis. The virulence was tested intravenously on rabbits and two calves. Localized chronic tuberculosis was produced in rabbits, but one calf developed a cold abscess on one leg, containing virulent tubercle bacilli after nineteen months.

"H38 B." Culture recovered June 18, 1907, directly from cold abscess in above-mentioned calf on Loeffler's beef serum. It exhibited no increased virulence for rabbits.

"$H38_{*}$"–"$H38_{**}$." Cultures recovered after the first and second passage through guinea-pigs of the original H38.

"H39." Isolated Oct. 3, 1906, directly from sputum, the source (Miss McC.) being a case of acutely progressive tuberculosis. The culture was not virulent for rabbits when given intravenously.

"H40." Isolated May 7, 1908, directly from sputum, the source (Miss F.) being a case of acutely progressive tuberculosis. No tests of virulence were made on rabbits.

"H41." Isolated Oct. 12, 1908, directly from sputum on Hesse's agar, the source (J. C. G.) being a case of acutely progressing tuberculosis in a luetic patient. No tests of virulence were made on rabbits.

"Bac. Tim." Bacillus timotheus (Moeller). A culture isolated from herd's grass; obtained indirectly from Professor Moeller of Berlin, Sept. 10, 1904.

Tuberculins, bacillus preparations, etc.

No. 130 O.T. Crude tuberculin. This was prepared from 4,450 cubic centimeters of broth cultures R1 tubercle

bacilli Nov. 5, 1904, after the formula of Koch's old tuberculin.

No. 141 O.T. Crude tuberculin. Ditto from 4,620 cubic centimeters Feb. 12, 1907.

No. 143 "T.P."=Tuberculin purificatum. This was prepared July 19, 1907, from 500 cubic centimeters old tuberculin from H33 cultures by precipitation with 1,000 cubic centimeters, ninety-five per cent alcohol. The precipitate washed with seventy per cent alcohol and dried yielded 5 grams.

No. 153 O.T. Crude tuberculin from broth cultures, H37 tubercle bacilli, Jan. 10, 1909.

No. 1 "T.C.R." Twelve and four-tenths grams dry pulverized H33 broth cultures, tubercle bacilli, extracted successively at room temperature with aq. dest., NaCl ten per cent, alcohol ninety-five per cent, ether and chloroform, twenty-four hours for each. Residue 5.875 gms. = T.C.R. No. 1. Oct. 31, 1905.

Bacillary extracts A; 12; 17; 20; 21. — The first filtrates per bougie from the extraction of R1 tubercle bacilli with .5 per cent $NaHCO_3$, five times the weight of the moist bacilli (see Further Notes on Differences in Precipitins, etc.).

Bacillary extracts H33; 16; 18; 19; 22; 23. — Ditto of H33 tubercle bacilli.

Bacillary extracts F; 13. — Ditto of R1 tubercle bacilli with N/NaCl.

Bacillary residues D; 12; H33. The bacillus sediments remaining after four extractions with .5 per cent $NaHCO_3$, and centrifugalization.

Bacillus emulsions (Koch). R1 tubercle bacilli pulverized; 1 gram to 100 cubic centimeters, centrifugalized. Supernatant diluted to 3,000, 5,000, and 10,000 cubic centimeters N/NaCl + phenol .25 per cent.

Water extract No. 144. Miscellaneous extracts from R1, H33, H37 tubercle bacilli; filtered per bougie; evaporated

at 70 to 80° C.; precipitated with two volumes alcohol ninety-five per cent; washed with seventy per cent alcohol and dried.

T.B. extract No. 148. Fourteen grams dry H37 tubercle bacilli, extracted twenty-four hours at 50° C. with 140 cubic centimeters N/NaCl and a little toluol; centrifugalized and filtered per bougie. One cubic centimeter = .0107 grams net, dry substance — NaCl. Oct. 19, 1907.

No. 148 A. T.B. extract 148 diluted with water.

Water extract 148 B. The washed residue from 148 boiled twenty-four hours in aq. dest.; filtered per bougie; concentrated on water bath.

Water extract 148 C. Ten and four-tenths grams dry H39 tubercle bacilli from serum-broth cultures extracted with aq. dest. twenty-four hours at 50° C., and again at 100° C.; mixed; filtered per bougie. Total filtrate 100.4 cubic centimeters; NaCl added to normal strength. Nov. 25, 1907.

Water extract 148 D. Thirty-four grams dry mixed R1 and H37 tubercle bacilli twice extracted with aq. dest. at 50° C. twenty-four hours, and once at 100° C. Both extracts evaporated separately at 50° C. and 100° C. respectively, and mixed. Total, one hundred cubic centimeters. Dec. 7, 1907.

Water extract 151 A. Twenty-three grams dry tubercle bacilli were extracted three times for twenty-four hours with aq. dest. and a little toluol on a water bath at 50° C., the successive extracts being combined and filtered per Berkefeld bougie. The total filtrate was 855 cubic centimeters and of neutral reaction. Quantities of 5 cubic centimeters evaporated to dryness gave an average constant weight of 1 cubic centimeter = .0046 gram. The whole was now evaporated to 252 cubic centimeters. Weight approximately 1 cubic centimeter = .0155 grams. April 30, 1908.

Water extract 151 B. The above tubercle bacilli residue

was boiled in fifty per cent glycerine water several hours, filtered, the residue washed on paper and dried. The filtrate was passed through the bougie.

Water extract 151 C. The washed and dried residue from 151 B weighed twelve grams. It was pulverized three days in a mill, boiled in an open flask twelve hours with aq. dest. and filtered per bougie. The filtrate showed a moderate flocculent ppt. when tested with acetic acid and heat, but it was not estimated by weight. Aug. 24, 1908.

151 Residue. Tubercle bacilli residue above, suspended in N/NaCl 200 cubic centimeters.

151 "Eu-globulin." Thirty cubic centimeters W.E. 151 C. pptd. by one-third saturation $(NH_3)_2\ SO_4$ and filtered; residue washed with one-third saturated $(NH_4)_2\ SO_4$; dissolved in 20 cubic centimeters H_2O; dialysed twenty-four hours. Bulk made up to 30 cubic centimeters with H_2O.

151 C. Residue. The first filtrate saturated with $(NH_4)_2\ SO_4$ crystals, filtered; dissolved in 20 cubic centimeters H_2O; dialysed twenty-four hours and made up to 30 cubic centimeters.

Extract emulsion 152. Twenty-four grams H37 pulverized tubercle bacilli, digested at 55° to 60° C. with 500 cubic centimeters aq. dest. twenty-four hours, and centrifugalized. A second extract with 200 cubic centimeters was made from the residue and was added to the first. The residue was finally boiled twelve hours in H_2O and the entire product mixed and centrifugalized. The supernatant extract, an opalescent emulsion, was used for testing anaphylaxis, and contained approximately .016 gram solids to 1 cubic centimeter. It contained a few suspended dead tubercle bacilli. Dec. 28, 1908.

Water extract 152 F. A portion of the extract emulsion 152 filtered per bougie.

First residue 152. An emulsion of the first extract and a small portion of the sediment.

Water extract 154. Three and nine-tenths grams dry H37 tubercle bacilli twice extracted with aq. dest. twenty-four hours at 50 to 55° C. Filtered per bougie. Total, 52 cubic centimeters. March 17, 1908.

Extract emulsion 154 X. Fourteen grams dry H37 tubercle bacilli digested twice with aq. dest. twenty-four hours at 55 to 62° C. Centrifugalized; combined extracts = 500 cubic centimeters. One cubic centimeter contained .014 gram dry substance. The supernatant extract contained many tubercle bacilli. NaCl was added to make .85 per cent. May 26, 1909.

Water extract 157 Tim. Twenty-two grams (moist) fresh broth cultures B. Timothei freed from broth, digested at 45 to 50° C. with aq. dest. 500 cubic centimeters eighteen hours, and again twenty-four hours; filtered per bougie. One cubic centimeter = .0015 gram dry substance. July 13, 1909.

Extract emulsion 157. A portion of the above not filtered but centrifugalized.

Water extract 158. Twenty-six grams R1 tubercle bacilli pulverized, digested with aq. dest. at 50° C. twenty-four hours twice. Centrifugalized; combined extracts = 740 cubic centimeters. Filtered per bougie. One cubic centimeter = .0085 gram dry substance. Aug. 12, 1909.

Extract emulsion 158. A portion of above (100 cubic centimeters) supernatant extracts unfiltered.

T.R. emulsion 158. Washed and dried residue from above re-pulverized, about 1.5 grams taken up in N/NaCl 150 cubic centimeters.

Water extract 159. Twelve grams dry H37 tubercle bacilli extracted as in 158. Total, 210 cubic centimeters. One cubic centimeter = .006 gram dry substance.

Extract emulsion 159. A portion of water extract 159 unfiltered but centrifugalized.

Water extract 160. Twenty-eight grams dry H37 tubercle bacilli extracted and filtered as usual. Total, 650 cubic centimeters. One cubic centimeter = .0108 gram dry substance.

Extract emulsion 161. Twenty-two grams dry H37 tubercle bacilli extracted and centrifugalized. Total, 440 cubic centimeters. One cubic centimeter = .03325 gram dry substance.

Water extract 162 (bovine). Fourteen and five-tenths grams dry B1 tubercle bacilli extracted and filtered as usual. Total, 290 cubic centimeters. One cubic centimeter = .00825 gram dry substance.

Extract emulsion 162 (bovine). A portion of water extract 162 unfiltered but centrifugalized.